PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification [6] : C12N 15/86, 7/00, 15/88, A61K 48/00 // C07K 14/47 | A2

(11) International Publication Number: WO 96/13597

(43) International Publication Date: 9 May 1996 (09.05.96)

(21) International Application Number: PCT/US95/14017

(22) International Filing Date: 27 October 1995 (27.10.95)

(30) Priority Data:
08/331,381 28 October 1994 (28.10.94) US

(60) Parent Application or Grant
(63) Related by Continuation
US 08/331,381 (CIP)
Filed on 28 October 1994 (28.10.94)

(71) Applicant (*for all designated States except US*): THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA [US/US]; 133 South 36th Street, Philadelphia, PA 19104-3246 (US).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): WILSON, James, M. [US/US]; 1350 N. Avignon Drive, Gladwyne, PA 19035 (US). FISHER, Krishna, J. [US/US]; 4006 Pine Street, Philadelphia, PA 19104 (US). CHEN, Shu-Jen [-/US]; 3901 Conshohocken Avenue, Philadelphia, PA 19131 (US). WEITZMAN, Matthew [GB/US]; 301 S. 19th Street #2A, Philadelphia, PA 19103 (US).

(74) Agents: BAK, Mary, E. et al.; Howson and Howson, Spring House Corporate Center, P.O. Box 457, Spring House, PA 19477 (US).

(81) Designated States: AL, AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, RO, RU, SD, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, LS, MW, SD, SZ, UG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: IMPROVED ADENOVIRUS AND METHODS OF USE THEREOF

(57) Abstract

A recombinant adenovirus and a method for producing the virus are provided which utilize a recombinant shuttle vector comprising adenovirus DNA sequence for the 5' and 3' cis-elements necessary for replication and virion encapsidation in the absence of sequence encoding viral genes and a selected minigene linked thereto, and a helper adenovirus comprising sufficient adenovirus gene sequences necessary for a productive viral infection. Desirably the helper gene is crippled by modifications to its 5' packaging sequences, which facilitates purification of the viral particle from the helper virus.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgystan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo			SE	Sweden
CH	Switzerland	KR	Republic of Korea	SI	Slovenia
CI	Côte d'Ivoire	KZ	Kazakhstan	SK	Slovakia
CM	Cameroon	LI	Liechtenstein	SN	Senegal
CN	China	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
CZ	Czech Republic	LV	Latvia	TJ	Tajikistan
DE	Germany	MC	Monaco	TT	Trinidad and Tobago
DK	Denmark	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	US	United States of America
FI	Finland	ML	Mali	UZ	Uzbekistan
FR	France	MN	Mongolia	VN	Viet Nam
GA	Gabon				

IMPROVED ADENOVIRUS AND METHODS OF USE THEREOF

This invention was supported by the National Institute of Health Grant No. P30 DK 47757. The United States government has rights in this invention.

Field of the Invention

The present invention relates to the field of vectors useful in somatic gene therapy and the production thereof.

Background of the Invention

Human gene therapy is an approach to treating human disease that is based on the modification of gene expression in cells of the patient. It has become apparent over the last decade that the single most outstanding barrier to the success of gene therapy as a strategy for treating inherited diseases, cancer, and other genetic dysfunctions is the development of useful gene transfer vehicles. Eukaryotic viruses have been employed as vehicles for somatic gene therapy. Among the viral vectors that have been cited frequently in gene therapy research are adenoviruses.

Adenoviruses are eukaryotic DNA viruses that can be modified to efficiently deliver a therapeutic or reporter transgene to a variety of cell types. Recombinant adenoviruses types 2 and 5 (Ad2 and Ad5, respectively), which cause respiratory disease in humans, are currently being developed for gene therapy. Both Ad2 and Ad5 belong to a subclass of adenovirus that are not associated with human malignancies. Recombinant adenoviruses are capable of providing extremely high levels of transgene delivery to virtually all cell types, regardless of the mitotic state. High tit rs (10^{13} plaque forming units/ml) of recombinant virus can be asily generated in 293 cells (the adenovirus equivalent

to retrovirus pa:kaging cell lines) and cryo-stored for extended periods without appreciable losses. The efficacy of this system in delivering a therapeutic transgene *in vivo* that complements a genetic imbalance has been demonstrated in anima. models of various disorders [Y. Watanabe, Atherosclerosis, 36:261-268 (1986); K. Tanzawa et al, FEBS Letters, 118(1):81-84 (1980); J.L. Golasten et al, New Engl. J. Med., 309(11983):288-296 (1983); S. Ishibashi et al, J. Clin. Invest., 92:883-893 (1993); and S. Ishibashi et al, J. Clin. Invest., 93:1885-1893 (1994)]. Indeed, a recombinant replication defective adenovirus encoding a cDNA for the cystic fibrosis transmembrane regulator (CFTR) has been approved for use in at least two human CF clinical trials [see, e.g., J. Wilson, Nature, 365:691-692 (Oct. 21, 1993)]. Further support of the safety of recombinant adenoviruses for gene therapy is the extensive experience of live adenovirus vaccines in human populations.

Human adenoviruses are comprised of a linear, approximately 36 kb double-stranded DNA genome, which is divided into 100 map units (m.u.), each of which is 360 bp in length. The DNA contains short inverted terminal repeats (ITR) at each end of the genome that are required for viral DNA replication. The gene products are organized into early (E1 through E4) and late (L1 through L5) regions, based on expression before or after the initiation of viral DNA synthesis [see, e.g., Horwitz, Virology, 2d edit., ed. B. N. Fields, Raven Press, Ltd. , New York (1990)].

The first-generation recombinant, replication-deficient adenoviruses which have been developed for gen therapy contain deleti ns of the entire E1a and part of the E1b regions. This replication-defectiv virus is grown n an adenovirus-transformed, complementation human

embryonic kidney cell line containing a functional adenovirus E1a gene which provides a transacting E1a protein, the 293 cell [ATCC CRL1573]. E1-deleted viruses are capable of replicating and producing infectious virus in the 293 cells, which provide E1a and E1b region gene products in *trans*. The resulting virus is capable of infecting many cell types and can express the introduced gene (providing it carries its own promoter), but cannot replicate in a cell that does not carry the E1 region DNA unless the cell is infected at a very high multiplicity of infection.

However, *in vivo* studies revealed transgene expression in these E1 deleted vectors was transient and invariably associated with the development of severe inflammation at the site of vector targeting [S. Ishibashi et al, J. Clin. Invest., 93:1885-1893 (1994); J. M. Wilson et al, Proc. Natl. Acad. Sci., USA, 85:4421-4424 (1988); J. M. Wilson et al, Clin. Bio., 3:21-26 (1991); M. Grossman et al, Som. Cell. and Mol. Gen., 17:601-607 (1991)]. One explanation that has been proposed to explain this finding is that first generation recombinant adenoviruses, despite the deletion of E1 genes, express low levels of other viral proteins. This could be due to basal expression from the unstimulated viral promoters or transactivation by cellular factors. Expression of viral proteins leads to cellular immune responses to the genetically modified cells, resulting in their destruction and replacement with nontransgene containing cells.

There yet remains a need in the art for the development of additional adenovirus vector constructs for gene therapy.

Summary of the Invention

In one aspect, the invention provides the components of a novel recombinant adenovirus production system. One component is a shuttle plasmid, pAdΔ, that comprises adenovirus cis-elements necessary for replication and virion encapsidation and is deleted of all viral genes. This vector carries a selected transgene under the control of a selected promoter and other conventional vector/plasmid regulatory components. The other component is a helper adenovirus, which alone or with a packaging cell line, supplies sufficient gene sequences necessary for a productive viral infection. In a preferred embodiment, the helper virus has been altered to contain modifications to the native gene sequences which direct efficient packaging, so as to substantially disable or "cripple" the packaging function of the help r virus or its ability to replicate.

In another aspect, the present invention provides a unique recombinant adenovirus, an AdΔ virus, produced by use of the components above. This recombinant virus comprises an adenovirus capsid, adenovirus cis-elements necessary for replication and virion encapsidation, but is deleted of all viral genes (i.e., all viral open reading frames). This virus particle carries a selected transgene under the control of a selected promoter and other conventional vector regulatory components. This AdΔ recombinant virus is characterized by high titer transgene delivery to a host cell and the ability to stably integrate the transgene into the host cell chromosome. In one embodiment, the virus carries as its transgene a reporter gene. Another embodiment of the recombinant virus contains a th rapeutic transgene.

In an ther aspect, the invention provides a method for producing the above-describ d recombinant AdΔ virus by co-transfecting a cell line (either a packaging c ll

line or a non-packaging cell line) with a shuttle vector or plasmid and a helper adenovirus as described above, wherein the transfected cell generates the AdΔ virus. The AdΔ virus is subsequently isolated and purified therefrom.

In yet a further aspect, the invention provides a method for delivering a selected gene to a host cell for expression in that cell by administering an effective amount of a recombinant AdΔ virus containing a therapeutic transgene to a patient to treat or correct a genetically associated disorder or disease.

Other aspects and advantages of the present invention are described further in the following detailed description of the preferred embodiments thereof.

Brief Description of the Figures

Fig. 1A is a schematic representation of the organization of the major functional elements that define the 5' terminus from Ad5 including an inverted terminal repeat (ITR) and a packaging/enhancer domain. The TATA box of the E1 promoter (black box) and E1A transcriptional start site (arrow) are also shown.

Fig. 1B is an expanded schematic of the packaging/enhancer region of Fig. 1A, indicating the five packaging (PAC) domains (A-repeats), I through V. The arrows indicate the location of PCR primers referenced in Figs. 9A and 9B below.

Fig. 2A is a schematic of shuttle vector pAdΔ.CMVLacZ containing 5' ITR from Ad5, followed by a CMV promoter/enhancer, a LacZ gene, a 3' ITR from Ad5, and remaining plasmid sequence from plasmid pSP72 backbone. Restriction endonuclease enzymes are represented by conv ntional designations in the plasmid constructs.

Fig. 2B is a schematic of the shuttle vector digested with EcoRI to release the modified AdΔ genome from the pSP72 plasmid backbone.

Fig. 2C is a schematic depiction of the function of the vector system. In the pre‿ence of an E1-deleted helper virus Ad.CBhpAP which encodes a reporter minigene for human placenta alkaline phosphatase (hpAP), the AdΔ.CMVLacZ genome is packaged into preformed virion capsids, distinguishable from the helper virions by the presence of the LacZ gene.

Figs. 3A to 3F [SEQ ID NO: 1] report the top DNA strand of the double-stranded plasmid pAdΔ.CMVLacZ. The complementary sequence may be readily obtained by one of skill in the art. The sequence includes the following components: 3' Ad ITR (nucleotides 607-28 of SEQ ID NO: 1); the 5' Ad ITR (nucleotides 5496-5144 of SEQ ID NO: 1); CMV promoter/enhancer (nucleotides 5117-4524 of SEQ ID NO: 1); SD/SA sequence (nucleotides 4507-4376 of SEQ ID NO: 1); LacZ gene (nucleotides 4320-845 of SEQ ID NO: 1); and a poly A sequence (nucleotides 837-639 of SEQ ID NO: 1).

Fig. 4A is a schematic of shuttle vector pAdΔc.CMVLacZ containing an Ad5 5' ITR and 3' ITR positioned head-to-tail, with a CMV enhancer/promoter-LacZ minigene immediately following the 5' ITR, follow d by a plasmid pSP72 (Promega) backbone. Restriction endonuclease enzymes are represented by conventional designations in the plasmid constructs.

Fig. 4B is a schematic depiction of the function of the vector system of Fig. 4A. In the presence of helper virus Ad.CBhpAP, the circular pADΔc.CMVLacZ shuttle vector sequenc is packaged into virion heads, distinguishabl from the helper virions by th presenc of the LacZ gen .

Figs. 5A to 5F [SEQ ID NO: 2] report the top DNA strand of the double-stranded vector pAdΔc.CMVLacZ. The complementary sequence may be readily obtained by one of skill in the art. The sequence includes the following components: 5' Ad ITR (nucleotides 600-958 of SEQ ID NO: 2); CMV promoter/enhancer (nucleotides 969-1563 of SEQ ID NO: 2); SD/SA sequence (nucleotides 1579-1711); LacZ gene (nucleotides 1762-5236 of SEQ ID NO: 2); poly A sequence (nucleotides 5245-5443 of SEQ ID NO: 2); and 3' Ad ITR (nucleotides 16-596 of SEQ ID NO: 2).

Fig. 6 is a schematic of shuttle vector pAdΔ.CBCFTR containing 5' ITR from Ad5, followed by a chimeric CMV enhancer/ß actin promoter enhancer, a CFTR gene, a poly-A sequence, a 3' ITR from Ad5, and remaining plasmid sequence from plasmid pSL1180 (Pharmacia) backbone. Restriction endonuclease enzymes are represented by conventional designations in the plasmid constructs.

Figs. 7A to 7H [SEQ ID NO: 3] report the top DNA strand of the double-stranded plasmid pAdΔ.CBCFTR. The complementary sequence may be readily obtained by one of skill in the art. The sequence includes the following components: 5' Ad ITR (nucleotides 9611-9254 of SEQ ID NO: 3); chimeric CMV enhancer/ß actin promoter (nucleotides 9241-8684 of SEQ ID NO: 3); CFTR gene (nucleotides 8622-4065 of SEQ ID NO: 3); poly A sequence (nucleotides 3887-3684 of SEQ ID NO: 3); and 3' Ad ITR (nucleotides 3652-3073 of SEQ ID NO: 3). The remaining plasmid backbone is obtained from pSL1180 (Pharmacia).

Fig. 8A illustrates the generation of 5' adenovirus terminal sequence that contained PAC domains I and II by PCR. See, arrows indicating righthand and lefthand (PAC II) PCR probes in Fig. 1B.

Fig. 8B illustrates the generation of 5' terminal sequence that contained PAC domains I, II, III and IV by PCR. See, arrows indicating righthand and lefthand (PAC IV) PCR probes in Fig. 1B.

Fig. 8C depicts the amplification products subcloned into the multiple cloning site of pAd.Link.1 (IHGT Vector Core) generating pAd.PACII (domains I and II) and pAd.PACIV (domains I, II, III, and IV) resulting in crippled helper viruses, Ad.PACII and Ad.PACIV with modified packaging (PAC) signals.

Fig. 9A is a schematic representation of the subcloning of a human placenta alkaline phosphatase reporter minigene containing the immediate early CMV enhancer/ promoter (CMV), human placenta alkaline phosphatase cDNA (hpAP), and SV40 polyadenylation signal (pA) into pAd.PACII to result in crippled helper virus vector pAdΔ.PACII.CMVhpAP. Restriction endonuclease enzymes are represented by conventional designations in the plasmid constructs.

Fig. 9B is a schematic representation of the subcloning of the same minigene of Fig. 9A into pAd.PACIV to result in crippled helper virus vector pAd.PACIV.CMV.hpAP.

Fig. 10 is a flow diagram summarizing the synthesis of an adenovirus-based polycation helper virus conjugate and its combination with a pAdΔ shuttle vector to result in a novel viral particle complex. CsCl band purified helper adenovirus was reacted with the heterobifunctional crosslinker sulfo-SMCC and the capsid protein fiber is labeled with the nucleophilic maleimide moiety. Free sulfhydryls were introduced onto poly-L-lysine using 2-iminothiolane-HCl and mixed with the labelled adenovirus, resulting in the helper virus conjugate Ad-pLys. A unique adenovirus-bas d particle is generated by purifying the Ad-pLys conjugate over a CsCl gradient to

remove unincorporated poly-L-lysine, followed by extensively dialyzing, adding shuttle plasmid DNAs to Ad-pLys and allowing the complex formed by the shuttle plasmid wrapped around Ad-pLys to develop.

Fig. 11 is a schematic diagram of pCCL-DMD, which is described in detail in Example 9 below.

Fig. 12A - 12P provides the continuous DNA sequence of pAdΔ.CMVmDys [SEQ ID NO:10].

Detailed Description of the Invention

The present invention provides a unique recombinant adenovirus capable of delivering transgenes to target cells, as well as the components for production of the unique virus and methods for the use of the virus to treat a variety of genetic disorders.

The AdΔ virus of this invention is a viral particle containing only the adenovirus cis-elements necessary for replication and virion encapsidation (i.e., ITRs and packaging sequences), but otherwise deleted of all adenovirus genes (i.e., all viral open reading frames). This virus carries a selected transgene under the control of a selected promoter and other conventional regulatory components, such as a poly A signal. The AdΔ virus is characterized by improved persistence of the vector DNA in the host cells, reduced antigenicity/immunogenicity, and hence, improved performance as a delivery vehicle. An additional advantage of this invention is that the AdΔ virus permits the packaging of very large transgenes, such as a full-length dystrophin cDNA for the treatment of the progressive wasting of muscle tissue characteristic of Duchenne Muscular Dystrophy (DMD).

This novel recombinant virus is produced by use of an adenovirus-based vector production system containing two components: 1) a shuttle vector that compris s adenovirus cis-elements necessary for r plication and

virion encapsidation and is deleted of all viral genes, which vector carries a reporter or therapeutic minigene and 2) a helper adenovirus which, alone or with a packaging cell line, is capable of providing all of the viral gene products necessary ıɔr a productive viral infection when co-transfected with the shuttle vector. Preferably, the helper virus is modified so that it do s not package itself efficiently. In this setting, it is desirably used in combination with a packaging cell lin that stably expresses adenovirus genes. The methods of producing this viral vector from these components include both a novel means of packaging of an adenoviral/transgene containing vector into a virus, and a novel method for the subsequent separation of the helper virus from the newly formed recombinant virus.

I. The Shuttle Vector

The shuttle vector, referred to as pAdΔ, is composed of adenovirus sequences, and transgene sequences, including vector regulatory control sequences.

A. The Adenovirus Sequences

The adenovirus nucleic acid sequences of the shuttle vector provide the minimum adenovirus sequences which enable a viral particle to be produced with the assistance of a helper virus. These sequences assist in delivery of a recombinant transgene genome to a target cell by the resulting recombinant virus.

The DNA sequences of a number of adenovirus types are available from Genbank, including type Ad5 [Genbank Accession No. M73260]. The adenovirus sequences may be obtained from any known adenovirus serotype, such as s rotypes 2, 3, 4, 7, 12 and 40, and further including any of the presently identified 41 human types [see, e.g., Horwitz, cited above]. Similarly adenovirus s known t infect other animals may also be employed in the

vector constructs of this invention. The selection of the adenovirus type is not anticipated to limit the following invention. A variety of adenovirus strains are available from the American Type Culture Collection, Rockville, Maryland, or available by request from a variety of commercial and institutional sources. In the following exemplary embodiment an adenovirus, type 5 (Ad5) is used for convenience.

However, it is desirable to obtain a variety of pAdΔ shuttle vectors based on different human adenovirus serotypes. It is anticipated that a library of such plasmids and the resulting AdΔ viral vectors would be useful in a therapeutic regimen to evade cellular, and possibly humoral, immunity, and lengthen the duration of transgene expression, as well as improve the success of repeat therapeutic treatments. Additionally the use of various serotypes is believed to produce recombinant viruses with different tissue targeting specificities. The absence of adenoviral genes in the AdΔ viral vector is anticipated to reduce or eliminate adverse CTL response which normally causes destruction of recombinant adenoviruses deleted of only the E1 gene.

Specifically, the adenovirus nucleic acid sequences employed in the pAdΔ shuttle vector of this invention are adenovirus genomic sequences from which all viral genes are deleted. More specifically, the adenovirus sequences employed are the cis-acting 5' and 3' inverted terminal repeat (ITR) sequences of an adenovirus (which function as origins of replication) and the native 5' packaging/enhancer domain, that contains sequences necessary for packaging linear Ad genomes and enhancer elements for the E1 promoter. These sequences ar the sequences n cessary for r plication and virion encapsidation. See, e.g., P. Hearing et al, J. Virol., 61(8):2555-2558 (1987); M. Grabl and P. Hearing, J.

Virol., 64(5): 2047-2056 (1990); and M. Grable and P. Hearing, J. Virol., 66(2):723-731 (1992).

According to this invention, the entire adenovirus 5' sequence containing the 5' ITR and packaging/enhancer region can be employed as the 5' adenovirus sequence in the pAdΔ shuttle vector. This left terminal (5') sequence of the Ad5 genome useful in this invention spans bp 1 to about 360 of the conventional adenovirus genome, also referred to as map units 0-1 of the viral genome. This sequence is provided herein as nucleotides 5496-5144 of SEQ ID NO: 1, nucleotides 600-958 of SEQ ID NO: 2; and nucleotides 9611-9254 of SEQ ID NO: 3, and generally is from about 353 to about 360 nucleotides in length. This sequence includes the 5' ITR (bp 1-103 of the adenovirus genome), and the packaging/enhancer domain (bp 194-358 of the adenovirus genome). See, Figs. 1A, 3, 5, and 7.

Preferably, this native adenovirus 5' region is employed in the shuttle vector in unmodified form. However, some modifications including deletions, substitutions and additions to this sequence which do not adversely effect its biological function may be acceptable. See, e.g., WO 93/24641, published December 9, 1993. The ability to modify these ITR sequences is within the ability of one of skill in the art. See, e.g., texts such as Sambrook et al, "Molecular Cloning. A Laboratory Manual.", 2d edit., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1989).

The 3' adenovirus sequences of the shuttle vector include the right terminal (3') ITR sequence of the adenoviral genome spanning about bp 35,353 - end of th ad novirus genome, or map units ~98.4-100. This sequence is provided her in as nucl otides 607-28 of SEQ ID NO: 1, nucle tides 16-596 of SEQ ID NO: 2; and nucl otid s 3652-3073 of SEQ ID NO: 3, and generally is

about 580 nucleotides in length. This entire sequence is desirably employed as the 3' sequence of an pAdΔ shuttle vector. Preferably, the native adenovirus 3' region is employed in the shuttle vector in unmodified form. However, some modifications to this sequence which do not adversely effect its biological function may be acceptable.

An exemplary pAdΔ shuttle vector of this invention, described below and in Fig. 2A, contains only those adenovirus sequences required for packaging adenoviral genomic DNA into a preformed capsid head. The pAdΔ vector contains Ad5 sequences encoding the 5' terminal and 3' terminal sequences (identified in the description of Fig. 3), as well as the transgene sequences described below.

From the foregoing information, it is expected that one of skill in the art may employ other equivalent adenovirus sequences for use in the AdΔ vectors of this invention. These sequences may include other adenovirus strains, or the above mentioned cis-acting sequences with minor modifications.

B. The Transgene

The transgene sequence of the vector and recombinant virus is a nucleic acid sequence or reverse transcript thereof, heterologous to the adenovirus sequence, which encodes a polypeptide or protein of interest. The transgene is operatively linked to regulatory components in a manner which permits transgene transcription.

The composition of the transgene sequence will depend upon the use to which the resulting virus will be put. For example, one type of transgene sequence includes a report r sequence, which upon expression produces a detectable signal. Such report r sequences includ without limitation an *E. coli* beta-galactosidase

(*LacZ*) cDNA, a human placental alkaline phosphatase gene and a green fluorescent protein gene. These sequences, when associated with regulatory elements which drive their expression, provide signals detectable by conventional means, e.g., ultraviolet wavelength absorbance, visible color change, etc.

Another type of transgene sequence includes a therapeutic gene which expresses a desired gene product in a host cell. These therapeutic nucleic acid sequences typically encode products for administration and expression in a patient *in vivo* or *ex vivo* to replace or correct an inherited or non-inherited genetic defect or treat an epigenetic disorder or disease. Such therapeutic genes which are desirable for the performance of gene therapy include, without limitation, a normal cystic fibrosis transmembrane regulator (CFTR) gene (see Fig. 7), a low density lipoprotein (LDL) gene [T. Yamamoto et al, Cell, 39:27-28 (November, 1984)], a DMD cDNA sequence [partial sequences available from GenBank, Accession Nos. M36673, M36671, [A. P. Monaco et al, Nature, 323:646-650 (1986)] and L06900, [Roberts et al, Hum. Mutat., 2:293-299 (1993)]] (Genbank), and a number of genes which may be readily selected by one of skill in the art. The selection of the transgene is not considered to be a limitation of this invention, as such selection is within the knowledge of the art-skilled.

C. Regulatory Elements

In addition to the major elements identified above for the pAdΔ shuttle vector, i.e., the adenovirus sequences and the transgene, the vector also includes conventional regulatory elements necessary to drive xpression of the transgene in a c ll transfected with th pAdΔ vector. Thus the vector contains a selected promoter which is linked to the transgene and located,

with the transgene, between the adenovirus sequences of the vector.

Selection of the promoter is a routine matter and is not a limitation of the pAdΔ vector itself. Useful promoters may be constitutive promoters or regulated (inducible) promoters, which will enable control of the amount of the transgene to be expressed. For example, a desirable promoter is that of the cytomegalovirus immediate early promoter/enhancer [see, e.g., Boshart et al, Cell, 41:521-530 (1985)]. This promoter is found at nucleotides 5117-4524 of SEQ ID NO: 1 and nucleotides 969-1563 of SEQ ID NO: 2. Another promoter is the CMV enhancer/chicken β-actin promoter (nucleotides 9241-8684 of SEQ ID NO: 3). Another desirable promoter includes, without limitation, the Rous sarcoma virus LTR promoter/enhancer. Still other promoter/enhancer sequences may be selected by one of skill in the art.

The shuttle vectors will also desirably contain nucleic acid sequences heterologous to the adenovirus sequences including sequences providing signals required for efficient polyadenylation of the transcript and introns with functional splice donor and acceptor sites (SD/SA). A common poly-A sequence which is employed in the exemplary vectors of this invention is that derived from the papovavirus SV-40 [see, e.g., nucleotides 837-639 of SEQ ID NO: 1; 5245-5443 of SEQ ID NO: 2; and 3887-3684 of SEQ ID NO: 3]. The poly-A sequence generally is inserted in the vector following the transgene sequences and before the 3' adenovirus sequences. A common intron sequence is also derived from SV-40, and is referred to as the SV-40 T intron sequence [see, e.g., nucleotides 4507-4376 of SEQ ID NO: 1 and 1579-1711 of SEQ ID NO: 2]. A pAdΔ shuttle vector of the present invention may also contain such an intron, desirably located between the

promoter/enhancer sequence and the transgene. Selection of these and other common vector elements are conventional and many such sequences are available [see, e.g., Sambrook et al, and references cited therein]. Examples of such regulatory sequences for the above are provided in the plasmid sequences of Figs. 3, 5 and 7.

The combination of the transgene, promoter/ enhancer, the other regulatory vector elements are referred to as a "minigene" for ease of reference herein. The minigene is preferably flanked by the 5' and 3' cis-acting adenovirus sequences described above. Such a minigene may have a size in the range of several hundred base pairs up to about 30 kb due to the absence of adenovirus early and late gene sequences in the vector. Thus, this AdΔ vector system permits a great deal of latitude in the selection of the various components of the minigene, particularly the selected transgene, with regard to size. Provided with the teachings of this invention, the design of such a minigene can be made by resort to conventional techniques.

II. The Helper Virus

Because of the limited amount of adenovirus sequence present in the AdΔ shuttle vector, a helper adenovirus of this invention must, alone or in concert with a packaging cell line, provide sufficient adenovirus gene sequences necessary for a productive viral infection. Helper viruses useful in this invention thus contain selected adenovirus gene sequences, and optionally a second reporter minigene.

Normally, the production of a recombinant adenovirus which utilizes helper adenovirus containing a full compl ment of adenoviral gen s r sults in recombinant virus contaminated by xcess production of th h lper virus. Thus, xt nsive purification of the viral vector

from the contaminating helper virus is required. However, the present invention provides a way to facilitate purification and reduce contamination by crippling the helper virus.

One preferred embodiment of a helper virus of this invention thus contains three components (A) modifications or deletions of the native adenoviral gene sequences which direct efficient packaging, so as to substantially disable or "cripple" the packaging function of the helper virus or its ability to replicate, (B) selected adenovirus genes and (C) an optional reporter minigene. These "crippled" helper viruses may also be formed into poly-cation conjugates as described below.

The adenovirus sequences forming the helper virus may be obtained from the sources identified above in the discussion of the shuttle vector. Use of different Ad serotypes as helper viruses enables production of recombinant viruses containing the ΔAd (serotype 5) shuttle vector sequences in a capsid formed by the other serotype adenovirus. These recombinant viruses are desirable in targeting different tissues, or evading an immune response to the ΔAd sequences having a serotype 5 capsid. Use of these different Ad serotype helper viruses may also demonstrate advantages in recombinant virus production, stability and better packaging.

A. *The Crippling Modifications*

A desirable helper virus used in the production of the adenovirus vector of this invention is modified (or crippled) in its 5' ITR packaging/enhancer domain, identified above. As stated above, the packaging/enhancer region contains sequences necessary for packaging linear adenovirus genomes ("PAC" sequenc s). More sp cifically, this sequence contains at least s ven distinct y t functionally redundant domains

that are required for efficient encapsidation of replicated viral DNA.

Within a stretch of nucleotide sequence from bp 194-358 of the Ad5 genome, five of these so-called A-repeats or PAC sequences are localized (see, Fig. 1B). PAC I is located at bp 241-248 of the adenovirus genome (on the strand complementary to nucleotides 5259-5246 of SEQ ID NO: 1). PAC II is located at bp 262-269 of the adenovirus genome (on the strand complementary to nucleotides 5238-5225 of SEQ ID NO: 1). PAC III is located at bp 304-311 of the adenovirus genome (on the strand complementary to nucleotides 5196-5183 of SEQ ID NO: 1). PAC IV is located at bp 314-321 of the adenovirus (on the strand complementary to nucleotides 5186-5172 of SEQ ID NO: 1). PAC V is located at bp 339-346 of the adenovirus (on the strand complementary to nucleotides 5171-5147 of SEQ ID NO: 1).

Corresponding sequences can be obtained from SEQ ID NO: 2 and 3. PAC I is located at nucleotides 837-851 of SEQ ID NO: 2; and on the strand complementary to nucleotides 9374-9360 of SEQ ID NO: 3. PAC II is located at nucleotides 859-863 of SEQ ID NO: 2; and on the strand complementary to nucleotides 9353-9340 of SEQ ID NO: 3. PAC III is located at nucleotides 901-916 of SEQ ID NO: 2; and on the strand complementary to nucleotides 9311-9298 of SEQ ID NO: 3. PAC IV is located at nucleotides 911-924 of SEQ ID NO: 2; and on the strand complementary to nucleotides 9301-9288 of SEQ ID NO: 3. PAC V is located at nucleotides 936-949 of SEQ ID NO: 2; and on the strand complementary to nucleotides 9276-9263 of SEQ ID NO: 3.

Table 1 below lists these five native Ad5 sequences and a consensus PAC sequence based on the similarities between an eight nucleic acid stretch within the five sequences. The consensus sequence contains two positions at which the nucleic acid may be A or T (A/T). The conventional single letter designations are used for the nucleic acids, as is known to the art.

Table 1

A-Repeat	Adenovirus Genome Base Pair Nos. & Nucleotide sequence	
I	241 248 TAG TAAATTTG GGC	[SEQ ID NO: 4]
II	262 269 AGT AAGATTTG GCC	[SEQ ID NO: 5]
III	304 311 AGT GAAATCTG AAT	[SEQ ID NO: 6]
IV	314 321 GAA TAATTTTG TGT	[SEQ ID NO: 7]
V	339 346 CGT AATATTTG TCT	[SEQ ID NO: 8]
Consensus	5' (A/T)AN(A/T)TTTG 3'	[SEQ ID NO: 9]

According to this invention, mutations or deletions may be made to one or more of these PAC sequences to generate desirable crippled helper viruses. A deletion analysis of the packaging domain revealed a positive correlation between encapsidation efficiency and the number of packaging A-repeats that were present at the 5' end of the genome. Modifications of this domain may include 5' adenovirus sequences which contain less than all five of the PAC sequences of Table 1. For example, only two PAC sequences may be present in the crippled virus, e.g., PAC I and PAC II, PAC III and PAC IV, and so on. Deletions of select d PAC sequences may

involve deletion of contiguous or non-contiguous sequences. For example, PAC II and PAC IV may be deleted, leaving PAC I, III and IV in the 5' sequence. Still an alternative modification may be the replacement of one or more of the native PAC sequences with one or more repeats of the consensus sequence of Table 1. Alternatively, this adenovirus region may be modified by deliberately inserted mutations which disrupt one or more of the native PAC sequences. One of skill in the art may further manipulate the PAC sequences to similarly achiev the effect of reducing the helper virus packaging efficiency to a desired level.

Exemplary helper viruses which involve the manipulation of the PAC sequences described above are disclosed in Example 7 below. Briefly, as described in that example, one helper virus contains in place of th native 5' ITR region (adenovirus genome bp 1-360), a 5' adenovirus sequence spanning adenovirus genome bp 1-269, which contains only the 5' ITR and PAC I and PAC II sequences, and deletes the adenovirus region bp 270-360.

Another PAC sequence modified helper virus contains only the 5' Ad5 sequence of the ITR and PAC I through PAC IV (Ad bp 1-321), deleting PAC V and other sequences in the Ad region bp322-360.

These modified helper viruses are characterized by reduced efficiency of helper virus encapsidation. These helper viruses with the specific modifications of the sequences related to packaging efficiency, provide a packaging efficiency high enough for generating production lots of the helper virus, yet low enough that they permit the achievement of higher yields of AdΔ transducing viral particles according to this invention.

B. The Selected Adenovirus Genes

Helper viruses useful in this invention, whether or not they contain the "crippling" modifications described above, contain selected adenovirus gene sequences depending upon the cell line which is transfected by the helper virus and shuttle vector. A preferred helper virus contains a variety of adenovirus genes in addition to the modified sequences described above.

As one example, if the cell line employed to produce the recombinant virus is not a packaging cell line, the helper virus may be a wild type Ad virus. Thus, the helper virus supplies the necessary adenovirus early genes E1, E2, E4 and all remaining late, intermediate, structural and non-structural genes of the adenovirus genome. This helper virus may be a crippled helper virus by incorporating modifications in its native 5' packaging/enhancer domain.

A desirable helper virus is replication defective and lacks all or a sufficient portion of the adenoviral early immediate early gene E1a (which spans mu 1.3 to 4.5) and delayed early gene E1b (which spans mu 4.6 to 11.2) so as to eliminate their normal biological functions. Such replication deficient viruses may also have crippling modifications in the packaging/enhancer domain. Because of the difficulty surrounding the absolute removal of adenovirus from AdΔ preparations that have been enriched by CsCl buoyant density centrifugation, the use of a replication defective adenovirus helper prevents the introduction of infectious adenovirus for *in vivo* animal studies. This helper virus is employed with a packaging cell line which supplies the defici nt E1 proteins, such as the 293 c ll line.

Additionally, all or a portion of the adenovirus delayed early gene E3 (which spans mu 76.6 to 86.2) may be eliminated from the adenovirus sequence which forms a part of the helper viruses useful in this invention, without adversely affecting the function of the helper virus because this gene product is not necessary for the formation of a functioning virus.

In the presence of other packaging cell lines which are capable of supplying adenoviral proteins in addition to the E1, the helper virus may accordingly be deleted of the genes encoding these adenoviral proteins. Such additionally deleted helper viruses also desirably contain crippling modifications as described above.

C. A Reporter Minigene

It is also desirable for the helper virus to contain a reporter minigene, in which the reporter gene is desirably different from the reporter transgene contained in the shuttle vector. A number of such reporter genes are known, as referred to above. The presence of a reporter gene on the helper virus which is different from the reporter gene on the pAdΔ, allows both the recombinant AdΔ virus and the helper virus to be independently monitored. For example, the expression of recombinant alkaline phosphatase enables residual quantities of contaminating adenovirus to be monitored independent of recombinant LacZ expressed by an pAdΔ shuttle vector or an AdΔ virus.

D. Helper Virus Polycation Conjugates

Still another method for reducing the contamination of helper virus involves the formation of poly-cation helper virus conjugates, which may be associated with a plasmid containing other adenoviral genes, which are not pres nt in the helper virus. The help r viruses described above may be further modified by r sort to adenovirus-polylysine conjugate technology.

See, e.g., Wu et al, J. Biol. Chem., 264:16985-16987 (1989); and K. J. Fisher and J. M. Wilson, Biochem. J., 299: 49 (April 1, 1994), incorporated herein by reference.

Using this technolog;', a helper virus containing preferably the late adenoviral genes is modified by the addition of a poly-cation sequence distributed around the capsid of the helper virus. Preferably, the poly-cation is poly-lysine, which attaches around the negatively-charged vector to form an external positive charge. A plasmid is then designed to express those adenoviral genes not present in the helper virus, e.g., the E1, E2 and/or E4 genes. The plasmid associates to the helper virus-conjugate through the charges on the poly-lysine sequence. This modification is also desirably made to a crippled helper virus of this invention. This conjugate (also termed a trans-infection particle) permits additional adenovirus genes to be removed from the helper virus and be present on a plasmid which does not become incorporated into the virus during production of the recombinant viral vector. Thus, the impact of contamination is considerably lessened.

III. Assembly of Shuttle Vector, Helper Virus and Production of Recombinant Virus

The material from which the sequences used in the pAdΔ shuttle vector and the helper viruses are derived, as well as the various vector components and sequences employed in the construction of the shuttle vectors, helper viruses, and AdΔ viruses of this invention, are obtained from commercial or academic sources based on previously published and described materials. These materials may also be btained from an individual patient or gen rated and selected using standard recombinant molecular cloning techniques known and practiced by those

skilled in the art. Any modification of existing nucleic acid sequences forming the vectors and viruses, including sequence deletions, insertions, and other mutations are also generated using standard techniques.

Assembly of the selected DNA sequences of the adenovirus, and the reporter genes or therapeutic genes and other vector elements into the pAdΔ shuttle vector using conventional techniques is described in Example 1 below. Such techniques include conventional cloning techniques of cDNA such as those described in texts [Sambrook et al, cited above], use of overlapping oligonucleotide sequences of the adenovirus genomes, polymerase chain reaction, and any suitable method which provides the desired nucleotide sequence. Standard transfection and co-transfection techniques are employed, e.g., $CaPO_4$ transfection techniques using the HEK 293 cell line. Other conventional methods employed in this invention include homologous recombination of the viral genomes, plaquing of viruses in agar overlay, methods of measuring signal generation, and the like. Assembly of any desired AdΔ vector or helper virus of this invention is within the skill of the art, based on the teachings of this invention.

A. Shuttle Vector

As described in detail in Example 1 below and with resort to Fig. 2A and the DNA sequence of the plasmid reported in Fig. 3, a unique pAdΔ shuttle vector of this invention, pAdΔ.CMVLacZ, is generated. pAdΔ.CMVLacZ contains Ad5 sequences encoding the 5' terminal followed by a CMV promoter/enhancer, a splice donor/splice acceptor sequence, a bacterial beta-galactosidase gene (LacZ), a SV-40 poly A sequence (pA), a 3' ITR from Ad5 and remaining plasmid sequ nce from plasmid pSP72 (Promega) backbone.

To generate the AdΔ genome which is incorporated in the vector, the plasmid pAdΔ.CMVLacZ must be must be digested with EcoRI to release the AdΔ.CMVLacZ genome, freeing the adenovirus ITRs and making them available targets for replication. Thus production of the vector is "restriction-dependent", i.e., requires restriction endonuclease rescue of the replication template. See, Fig. 2B.

A second type of pAdΔ plasmid was designed which places the 3' Ad terminal sequence in a head-to-tail arrangement relative to the 5' terminal sequence. As described in Example 1 and Figs. 4A, and with resort to the DNA sequence of the plasmid reported in Fig. 5, a second unique AdΔ vector sequence of this invention, AdΔc.CMVLacZ, is generated from the shuttle plasmid pAdΔc.CMVLacZ, which contains an Ad5 5' ITR sequence and 3' ITR sequence positioned head-to-tail, followed by a CMV enhancer/ promoter, SD/SA sequence, LacZ gene and pA sequence in a plasmid pSP72 (Promega) backbone. As described in Example 1B, this "restriction-independent" plasmid permits the AdΔ genome to be replicated and rescued from the plasmid backbone without including an endonuclease treatment (see, Fig. 4B).

B. Helper Virus

As described in detail in Example 2, an exemplary conventional E1 deleted adenovirus helper virus is virus Ad.CBhpAP, which contains a 5' adenovirus sequence from mu 0-1, a reporter minigene containing human placenta alkaline phosphatase (hpAP) under the transcriptional control of the chicken β-actin promoter, followed by a poly-A sequence from SV40, followed by adenovirus sequences from 9.2 to 78.4 and 86 to 100. This helper contain d d letions from mu 1.0 to 9.2 and 78.4 to 86, which liminate substantially the E1 region and the E3 region of the virus. This virus may be

desirably crippled according to this invention by modifications to its packaging enhancer domain.

Exemplary crippled helper viruses of this invention are described using the techniques described in Example 7 and contain the modiiied 5' PAC sequences, i.e., adenovirus genome bp 1-269; m.u. 0-0.75 or adenovirus genome bp 1-321; m.u. 0-0.89. Briefly, the 5' sequences are modified by PCR and cloned by conventional techniques into a conventional adenovirus based plasmid. A hpAP minigene is incorporated into the plasmid, which is then altered by homologous recombination with an E3 deleted adenovirus dl7001 to result in the modified vectors so that the reporter minigene is followed on its 3' end with the adenovirus sequences mu 9.6 to 78.3 and 87 to 100.

Generation of a poly-L-lysine conjugate help r virus was demonstrated essentially as described in detail in Example 5 below and Fig. 10 by coupling poly-L-lysine to the Ad.CBhpAP virion capsid. Alternatively, the same procedure may be employed with the PAC sequence modifi d helper viruses of this invention.

C. Recombinant AdΔ Virus

As stated above, a pAdΔ shuttle vector in the presence of helper virus and/or a packaging cell line permits the adenovirus-transgene sequences in the shuttle vector to be replicated and packaged into virion capsids, resulting in the recombinant AdΔ virus. The current method for producing such AdΔ virus is transfection-based and described in detail in Example 3. Briefly, helper virus is used to infect cells, such as the packaging cell line human HEK 293, which are then subsequently transf ct d with an pAdΔ shuttle vector containing a selected transgene by conventional methods. About 30 or more hours post-transfection, th cells are harvested, and an xtract prepared. Th AdΔ viral g nome is

packaged into virions that sediment at a lower density than the helper virus in cesium gradients. Thus, the recombinant AdΔ virus containing a selected transgene is separated from the bulk of the helper virus by purification via buoyant density ultracentrifugation in a CsCl gradient.

The yield of AdΔ transducing virus is largely dependent on the number of cells that are transfected with the pAdΔ shuttle plasmid, making it desirable to use a transfection protocol with high efficiency. One such method involves use of a poly-L-lysinylated helper adenovirus as described above. A pAdΔ shuttle plasmid containing the desired transgene under the control of a suitable promoter, as described above, is then complexed directly to the positively charged helper virus capsid, resulting in the formation of a single transfection particle containing the pAdΔ shuttle vector and the helper functions of the helper virus.

The underlying principle is that the helper adenovirus coated with plasmid pAdΔ DNA will co-transport the attached nucleic acid across the cell membrane and into the cytoplasm according to its normal mechanism of cell entry. Therefore, the poly-L-lysine modified helper adenovirus assumes multiple roles in the context of an AdΔ-based complex. First, it is the structural foundation upon which plasmid DNA can bind increasing the effective concentration. Second, receptor mediated endocytosis of the virus provides the vehicle for cell uptake of the plasmid DNA. Third, the endosomalytic activity associated with adenoviral infection facilitates the release of internalized plasmid into the cytoplasm. And the adenovirus contributes trans helper functions on which the r combinant AdΔ virus is dependent for replication and packaging of transducing viral particles. The Ad-based transfection procedure using an pAdΔ shuttle

vector and a polycation-helper conjugate is detailed in Example 6. Additionally, as described previously, the helper virus-plasmid conjugate may be another form of helper virus delivery of the omitted adenovirus genes not present in the pAdΔ vector. Such a structure enables the rest of the required adenovirus genes to be divided between the plasmid and the helper virus, thus reducing the self-replication efficiency of the helper virus.

A presently preferred method of producing the recombinant AdΔ virus of this invention involves performing the above-described transfection with the crippled helper virus or crippled helper virus conjugate, as described above. A "crippled" helper virus of this invention is unable to package itself efficiently, and therefor permits ready separation of the helper virus from the newly packaged AdΔ vector of this invention by use of buoyant density ultracentrifugation in a CsCl gradient, as described in the examples below.

IV. Function of the Recombinant AdΔ Virus

Once the AdΔ virus of this invention is produced by cooperation of the shuttle vector and helper virus, the AdΔ virus can be targeted to, and taken up by, a selected target cell. The selection of the target cell also depends upon the use of the recombinant virus, i.e., whether or not the transgene is to be replicated *in vitro* or *ex vivo* for production in a desired cell type for redelivery into a patient, or *in vivo* for delivery to a particular cell type or tissue. Target cells may be any mammalian cell (preferably a human cell). For example, in *in vivo* use, the recombinant virus can target to any c ll type normally infected by adenovirus, depending upon the route of administration, i.e., it can targ t, without limitation, neurons, hepatocytes, epithelial cells and

the like. The helper adenovirus sequences supply the sequences necessary to permit uptake of the virus by the AdΔ.

Once the recombinant virus is taken up by a cell, the adenovirus flanked transgene is rescued from the parental adenovirus backbone by the machinery of the infected cell, as with other recombinant adenoviruses. Once uncoupled (rescued) from the genome of the AdΔ virus, the recombinant minigene seeks an integration site in the host chromatin and becomes integrated therein, either transiently or stably, providing expression of th accompanying transgene in the host cell.

V. Use of the AdΔ Viruses in Gene Therapy

The novel recombinant viruses and viral conjugates of this invention provide efficient gene transfer vehicles for somatic gene therapy. These viruses are prepared to contain a therapeutic gene in place of the *LacZ* reporter transgene illustrated in the exemplary viruses and vectors. By use of the AdΔ viruses containing therapeutic transgenes, these transgenes can be delivered to a patient *in vivo* or *ex vivo* to provide for integration of the desired gene into a target cell. Thus, these viruses can be employed to correct genetic deficiencies or defects. An example of the generation of an AdΔ gene transfer vehicle for the treatment of cystic fibrosis is described in Example 4 below. One of skill in the art can generate any number of other gene transfer vehicles by including a selected transgene for the treatment of other disorders.

The recombinant viruses of the present invention may be administered to a patient, preferably suspended in a biologically compatible solution or pharmac utically acceptabl delivery vehicle. A suitabl v hicle includes sterile saline. Other aqueous and non-aqueous isotonic

sterile injection solutions and aqueous and non-aqueous sterile suspensions known to be pharmaceutically acceptable carriers and well known to those of skill in the art may be employed for this purpose.

The recombinant viruses of this invention may be administered in sufficient amounts to transfect the desired cells and provide sufficient levels of integration and expression of the selected transgene to provide a therapeutic benefit without undue adverse effects or with medically acceptable physiological effects which can be determined by those skilled in the medical arts. Conventional and pharmaceutically acceptable parenteral routes of administration include direct delivery to the target organ, tissue or site, intranasal, intravenous, intramuscular, subcutaneous, intradermal and oral administration. Routes of administration may be combined, if desired.

Dosages of the recombinant virus will depend primarily on factors such as the condition being treated, the selected gene, the age, weight and health of the patient, and may thus vary among patients. A therapeutically effective human dosage of the viruses of the present invention is believed to be in the range of from about 20 to about 50 ml of saline solution containing concentrations of from about 1×10^7 to 1×10^{10} pfu/ml virus of the present invention. A preferr d human dosage is about 20 ml saline solution at the above concentrations. The dosage will be adjusted to balance the therapeutic benefit against any side effects. The levels of expression of the selected gene can be monitored to determine the selection, adjustment or frequency of dosag administration.

The following examples illustrate the construction of the pAdΔ shuttle vectors, helper viruses and recombinant AdΔ viruses of the present invention and the use thereof in gene therapy. These examples are illustrative only, and do not limit the scope of the present invention.

Example 1 - Production of pAdΔ.CMVLacZ and pAdΔc.CMVLacZ Shuttle Vectors

A. pAdΔ.CMVLacZ

A human adenovirus Ad5 sequence was modified to contain a deletion in the E1a region [map units 1 to 9.2], which immediately follows the Ad 5' region (bp 1-360) (illustrated in Figs. 1A). Thus, the plasmid contains the 5' ITR sequence (bp 1-103), the native packaging/enhancer sequences and the TATA box for the E1a region (bp 104-360). A minigene containing the CMV immediate early enhancer/promoter, an SD/SA sequence, a cytoplasmic lacZ gene, and SV40 poly A (pA), was introduced at the site of the E1a deletion. This construct was further modified so that the minigene is followed by the 3' ITR sequences (bp 35,353-end). The DNA sequences for these components are provided in Fig. 3 and SEQ ID NO: 1 (see, also the brief description of this figure).

This construct was then cloned by conventional techniques into a pSP72 vector (Promega) backbone to make the circular shuttle vector pAdΔCMVLacZ. See the schematic of Fig. 2A. This construct was engineered with EcoRI sites flanking the 5' and 3' Ad5 ITR sequences. pAdΔ.CMVLacZ was then subjected to enzymatic digestion with EcoRI, releasing a linear fragment of the vector spanning the terminal end of the Ad 5'ITR sequence through the terminal end of the 3'ITR sequ nce from the plasmid backbone. Se Fig. 2B.

B. pAdΔc.CMVLacZ

The shuttle vector pAdΔc.CMVLacZ (Figs. 4A and 5) was constructed using a pSP72 (Promega) backbone so that the Ad5 5' ITR and 3' ITR were positioned head-to-tail. The organization of the Ad5 ITRs was based on reports that suggest circular Ad genomes that have the terminal ends fused together head-to-tail are infectious to levels comparable to linear Ad genomes. A minigene encoding the CMV enhancer, an SD/SA sequence, the LacZ gene, and the poly A sequence was inserted immediately following the 5' ITR. The DNA sequence of the resulting plasmid and the sequences for the individual components are reported in Fig. 5 and SEQ ID NO: 2 (see also, brief description of Fig. 5). This plasmid does not require enzymatic digestion prior to its use to produce the viral particle (see Example 3). This vector was designed to enable restriction-independent production of LacZ AdΔ vectors.

Example 2 - Construction of a Helper Virus

The Ad.CBhpAP helper virus [K. Kozarsky et al, Som. Cell Mol. Genet., 19(5):449-458 (1993)] is a replication deficient adenovirus containing an alkaline phosphatase minigene. Its construction involved conventional cloning and homologous recombination techniques. The adenovirus DNA substrate was extracted from CsCl purified d17001 virions, an Ad5 (serotype subgroup C) variant that carries a 3 kb deletion between mu 78.4 through 86 in the nonessential E3 region (provided by Dr. William Wold, Washington University, St. Louis, Missouri). Viral DNA was prepared for co-transfection by digestion with ClaI (adenovirus g nomic bp position 917) which r mov s the left arm of the genome encompassing adenovirus map units 0-2.5. S e lower diagram of Fig. 1B.

A parental cloning vector, pAd.BglII was designed. It contains two segments of wild-type Ad5 genome (i.e., map units 0-1 and 9-16.1) separated by a unique BglII cloning site for insertion of heterologous sequences. The missing Ad5 sequences betw.een the two domains (adenovirus genome bp 361-3327) results in the deletion of E1a and the majority of E1b following recombination with viral DNA.

A recombinant hpAP minigene was designed and inserted into the BglII site of pAd.BglII to generate the complementing plasmid, pAdCBhpAP. The linear arrangement of this minigene includes:

(a) the chicken cytoplasmic ß-actin promoter [nucleotides +1 to +275 as described in T. A. Kost et al, Nucl. Acids Res., 11(23):8287 (1983); nucleotides 9241-8684 of Fig. 7];

(b) an SV40 intron (e.g., nucleotides 1579-1711 of SEQ ID NO: 2),

(c) the sequence for human placental alkaline phosphatase (available from Genbank) and

(d) an SV40 polyadenylation signal (a 237 Bam HI-BclI restriction fragment containing the cleavage/poly-A signals from both the early and late transcription units; e.g., nucleotides 837-639 of SEQ ID NO: 1).

The resulting complementing plasmid, pAdCBhpAP contained a single copy of recombinant hpAP minigene flanked by adenovirus coordinates 0-1 on one side and 9.2-16.1 on the other.

Plasmid DNA was linearized using a unique NheI site immediately 5' to adenovirus map unit zero (0) and the above-identified adenovirus substrate and the complementing plasmid DNAs were transfected to 293 cells [ATCC CRL1573] using a standard calcium phosphate transfection procedure [see, .g., Sambrook et al, cited abov]. The end r sult of homologous recombination

involving sequences that map to adenovirus map units 9-16.1 is hybrid Ad.CBhpAP helper virus which contains adenovirus map units 0-1 and, in place of the E1a and E1b coding regions from the dl7001 adenovirus substrate, is the hpAP minigene from the plasmid, followed by Ad sequences 9 to 100, with a deletion in the E3 (78.4-86 mu) regions.

Example 3 - Production of Recombinant AdΔ Virus

The recombinant AdΔ virus of this invention are generated by co-transfection of a shuttle vector with th helper virus in a selected packaging or non-packaging cell line.

As described in detail below, the linear fragment provided in Example 1A, or the circular AdΔ genome carrying the LacZ of Example 1B, is packaged into the Ad.CBhpAP helper virus (Example 2) using conventional techniques, which provides an empty capsid head, as illustrated in Fig. 2C. Those virus particles which have successfully taken up the pAd shuttle genome into the capsid head can be distinguished from those containing the hpAP gene by virtue of the differential expression of LacZ and hpAP.

In more detail, 293 cells (4 x 10^7 pfu 293 cells/150 mm dish) were seeded and infected with helper virus Ad.CBhpAP (produced as described in Example 2) at an MOI of 5 in 20 ml DMEM/2% fetal bovine serum (FBS). This helper specific marker is critical for monitoring the level of helper virus contamination in AdΔ preparations before and after purification. The helper virus provides in trans the necessary helper functions for synthesis and packaging of the AdΔCMV*LacZ* genome.

Two hours post infecti n, using either the restriction-dep ndent shuttle vector or the restriction-independent shuttle vector, plasmid pAdΔ.CMVLacZ

(digested with EcoRI) or pAdΔc.CMVLacZ DNA, each carrying a LacZ minigene, was added to the cells by a calcium phosphate precipitate (2.5 ml calcium phosphate transfection cocktail containing 50 μg plasmid DNA).

Thirty to forty hours post-transfection, cells were harvested, suspended in 10 mM Tris-Cl (pH 8.0) (0.5 ml/150 mm plate) and frozen at -80°C. Frozen cell suspensions were subjected to three rounds of freeze (ethanol-dry ice)-thaw (37°C) cycles to release virion capsids. Cell debris was removed by centrifugation (5,000xg for 10 minutes) and the clarified supernatant applied to a CsCl gradients to separate recombinant virus from helper virus as follows.

Supernatants (10 ml) applied to the discontinuous CsCl gradient (composed of equal volumes of CsCl at 1.2 g/ml, 1.36 g/ml, and 1.45 g/ml 10 mM Tris-Cl (pH 8.0)) were centrifuged for 8 hours at 72,128Xg, resulting in separation of infectious helper virus from incompletely formed virions. Fractions were collected from the interfacing zone between the helper and top components and analyzed by Southern blot hybridization or for the presence of LacZ transducing particles. For functional analysis, aliquots (2.0 ml from each sample) from the same fractions were added to monolayers of 293 cells (in 35 mm wells) and expression of recombinant ß-galactosidase determined 24 hours later. More specifically, monolayers were harvested, suspended in 0.3 ml 10 mM Tris-Cl (pH 8.0) buffer and an extract prepared by three rounds of freeze-thaw cycles. Cell debris was removed by centrifugation and the supernatant tested for ß-galactosidase (LacZ) activity according to the procedure described in J. Price et al, Proc. Natl. Acad. Sci., USA, 84:156-160 (1987). Th specific activity (milliunits ß-galactosidase/mg prot in or reporter

enzymes was measured from indicator cells. For the recombinant virus, specific activity was 116.

Fractions with ß-galactosidase activity from the discontinuous gradient were sedimented through an equilibrium cesium gradient to further enrich the preparation for Ad∆ virus. A linear gradient was generated in the area of the recombinant virus spanning densities 1.29 to 1.34gm/ml. A sharp peak of the recombinant virus, detected as the appearance of the ß-gal activity in infected 293 cells, eluted between 1.31 and 1.33 gm/dl. This peak of recombinant virus was located between two major A_{260} nm absorbing peaks and in an area of the gradient with the helper virus was precipitously dropping off. The equilibrium sedimentation gradient accomplished another 102 to 103 fold purification of recombinant virus from helper virus. The yield of recombinant Ad∆.CMVLacZ virus recovered from a 50 plate prep after 2 sedimentations ranged from 107 to 108 transducing particles.

Analysis of lysates of cells transfected with the recombinant vector and infected with helper revealed virions capable of transducing the recombinant minigene contained within the vector. Subjecting aliquots of the fractions to Southern analysis using probes specific to the recombinant virus or helper virus revealed packaging of multiple molecular forms of vector derived sequence. The predominant form of the deleted viral genome was the size (~5.5 kb) of the corresponding double stranded DNA monomer (Ad∆.CMVLacZ) with less abundant but discrete higher molecular weight species (~10 kb and ~15 kb) also present. Full-length helper virus is 35kb. Importantly, the peak of vector transduction activity corresponds with th highest mol cular weight form of the delet d virus. These results confirm the hypothesis that ITRs and contiguous packaging sequence are the only el ments

necessary for incorporation into virions. An apparently ordered or preferred rearrangement of the recombinant Ad monomer genome leads to a more biologically active molecule. The fact that larger molecular species of the deleted genome are 2x and 3x fold larger than the monomer deleted virus genome suggests that the rearrangements may involve sequential duplication of the original genome.

These same procedures may be adapted for production of a recombinant AdΔ virus using a crippled helper virus or helper virus conjugate as described previously.

Example 4 - Recombinant AdΔ Virus Containing a Therapeutic Minigene

To test the versatility of the recombinant AdΔ virus system, the reporter LacZ minigene obtained from pAdΔCMVLacZ was cassette replaced with a therapeutic minigene encoding CFTR.

The minigene contained human CFTR cDNA [Riordan et al, Science, 245:1066-1073 (1989); nucleotides 8622-4065 of SEQ ID NO: 3] under the transcriptional control of a chimeric CMV enhancer/chicken ß-actin promotor element (nucleotides +1 to +275 as described in T. A. Kost et al, Nucl. Acids Res., 11(23):8287 (1983); nucleotides 9241-8684 of SEQ ID NO: 3, Fig. 7); and followed by an SV-40 poly-A sequence (nucleotides 3887-3684 of SEQ ID NO: 3, Fig. 7).

The CFTR minigene was inserted into the E1 deletion site of an Ad5 virus (called pAd.E1Δ) which contains a deletion in E1a from mu 1-9.2 and a deletion in E3 from mu 78.4-86.

The resulting shuttle vector called pAdΔ.CBCFTR (see Figs. 6 and the DNA sequence of Fig. 7 [SEQ ID NO: 3]) used the same Ad ITRs of pAdΔCMVLacZ, but the Ad5 sequences terminated with NheI sites instead of EcoRI.

Therefore release of the minigene from the plasmid was accomplished by digestion with NheI.

The vector production system described in Example 3 was employed, using the helper virus Ad.CBhpAP (Example 2). Monolayers of 293 cells grown to 80-90% confluency in 150 mm culture dishes were infected with the helper virus at an MOI of 5. Infections were done in DMEM supplemented with 2% FBS at 20 ml media/150 mm plate. Two hours post-infection, 50 μg plasmid DNA in 2.5 ml transfection cocktail was added to each plate and evenly distributed.

Delivery of the pAdΔ.CBCFTR plasmid to 293 cells was mediated by formation of a calcium phosphate precipitate and AdΔ.CBCFTR virus resolved from Ad.CBhpAP helper virus by CsCl buoyant density ultracentrifugation as follows:

Cells were left in this condition for 10-14 h, afterwhich the infection/transfection media was replaced with 20 ml fresh DMEM/2% FBS. Approximately 30 h post-transfection, cells were harvested, suspended in 10 mM Tris-Cl (pH 8.0) buffer (0.5 ml/150 mm plate), and stored at -80°C.

Frozen cell suspensions were lysed by three sequential rounds of freeze (ethanol-dry ice)-thaw (37°C). Cell debris was removed by centrifugation (5,000 x g for 10 min) and 10 ml clarified extract layered onto a CsCl step gradient composed of three 9.0 ml tiers with densities 1.45 g/ml, 1.36 g/ml, and 1.20 g/ml CsCl in 10 mM Tris-Cl (pH 8.0) buffer. Centrifugation was performed at 20,000 rpm in a Beckman SW-28 rotor for 8 h at 4°C. Fractions (1.0 ml) were collected from the bottom of the centrifuge tube and analyzed for rΔAd transducing vectors. P ak fractions w re combined and banded to equilibrium. Fractions containing transducing virions were dialyzed against 20 mM HEPES (pH 7.8)/150 mM NaCl

(HBS) and stored frozen at -80°C in the presence of 10% glycerol or as a liquid stock at -20°C (HBS+40% glycerol).

Fractions collected after ultracentrifugation were analyzed for transgene expression and vector DNA. For *lacZ* ΔrAd vectors, 2 µl aliquots were added to 293 cell monolayers seeded in 35 mm culture wells. Twenty-four hours later cells were harvested, suspended in 0.3 ml 10 mM Tris-Cl (pH 8.0) buffer, and lysed by three rounds of freeze-thaw. Cell debris was removed by centrifugation (15,000 x g for 10 min) and assayed for total protein [Bradford, (1976)] and β-galactosidase activity [Sambrook et al, (1989)] using ONPG (o-Nitrophenyl β-D-galactopyranoside) as substrate.

Expression of CFTR protein from the AdΔ.CBCFTR vector was determined by immunofluorescence localization. Aliquots of AdΔ.CBCFTR, enriched by two-rounds of ultracentrifugation and exchanged to HBS storage buffer, were added to primary cultures of airway epithelial cells obtained from the lungs of CF transplant recipients. Twenty-four hours after the addition of vector, cells were harvested and affixed to glass slides using centrifugal force (Cytospin 3, Shandon Scientific Limited). Cells were fixed with freshly prepared 3% paraformaldehyde in PBS (1.4 mM KH_2PO_4, 4.3 mM Na_2HPO_4, 2.7 mM KCl, and 137 mM NaCl) for 15 min at room temperature (RT), washed twice in PBS, and permeabilized with 0.05% NP-40 for 10 min at RT. The immunofluorescence procedure began with a blocking step in 10% goat serum (PBS/GS) for 1 h at RT, followed by binding of the primary monoclonal mouse anti-human CFTR (R-domain specific) antibody (Genzyme) diluted 1:500 in PBS/GS for 2 h at RT. Cells were washed extensively in PBS/GS and incubated for 1 h at RT with a donkey anti-mouse IgG (H+L) FITC conjugated

antibody (Jackson ImmunoResearch Laboratories) diluted 1:100 in PBS/GS.

For Southern analysis of vector DNA, 5 μl aliquots were taken directly from CsCl fractions and incubated with 20 μl capsid digestion buffer (50 mM Tris-Cl, pH 8.0; 1.0 mM EDTA, pH 8.0; 0.5% SDS, and 1.0 mg/ml Proteinase K) at 50°C for 1 h. The reactions were allowed to cool to RT, loading dye was added, and electrophoresed through a 1.2% agarose gel. Resolved DNAs were electroblotted onto a nylon membrane (Hybond-N) and hybridized with a 32-P labeled restriction fragment. Blots were analyzed by autoradiography or scanned on a Phosphorimager 445 SI (Molecular Dynamics).

The results that were obtained from Southern blot analysis of gradient fractions revealed a distinct viral band that migrated faster than the helper Ad.CBhpAP DNA. The highest viral titers mapped to fractions 3 and 4. Quantitation of the bands in fraction 4 indicated the titer of Ad.CBhpAP was approximately 1.5x greater than AdΔCBCFTR. However, if the size difference between the two viruses is factored in (Ad.CBhpAP=35 kb; AdΔCBCFTR=6.2 kb), the viral titer (where 1 particle=1 DNA molecule) of AdΔCB.CFTR is at least 4-fold greater than the viral titer of Ad.CBhpAP.

While Southern blot analysis of gradient fractions was useful for showing the production of AdΔ viral particles, it also demonstrated the utility of ultracentrifugation for purifying AdΔ viruses. Considering the latter of these, both LacZ and CFTR transducing viruses banded in CsCl to an intermediate density between infectious adenovirus helper virions (1.34 g/ml) and incomplet ly formed capsids (1.31 g/ml). Th lighter density relative to help r virus likely results from the smaller genome carri d by the AdΔ viruses. This further suggests chang s in virus size

influences the density and purification of AdΔ virus. Regardless, the ability to separate AdΔ virus from the helper virus is an important observation and suggests further purification may be achieved by successive rounds of banding through CsCl.

This recombinant virus is useful in gene therapy alone, or preferably, in the form of a conjugate prepared as described herein.

Example 5 - Correction of Genetic Defect in CF airway Epithelial Cells with AdΔCB.CFTR

Treatment of cystic fibrosis, utilizing the recombinant virus provided above, is particularly suited for *in vivo*, lung-directed, gene therapy. Airway epithelial cells are the most desirable targets for gene transfer because the pulmonary complications of CF are usually its most morbid and life-limiting.

The recombinant AdΔCB.CFTR virus was fractionated on sequential CsCl gradients and fractions containing CFTR sequences, migrating between the adenovirus and top components fractions described above were used to infect primary cultures of human airway epithelial cells derived from the lungs of a CF patient. The cultures were subsequently analyzed for expression of CFTR protein by immunocytochemistry. Immunofluorescent detection with mouse anti-human CFTR (R domain specific) antibody was performed 24 hours after the addition of the recombinant virus. Analysis of mock infected CF cells failed to reveal significant binding to the R domain specific CFTR antibody. Primary airway epithelium cultures exposed to the recombinant virus demonstrated high levels of CFTR protein in 10-20% of the cells.

Thus, the recombinant virus of the invention, containing the CFTR gene, may be delivered directly into the airway, e.g. by a formulating the virus above, into a

preparation which can be inhaled. For example, the recombinant virus or conjugate of the invention containing the CFTR gene, is suspended in 0.25 molar sodium chloride. The virus or conjugate is taken up by respiratory airway cells and the gene is expressed.

Alternatively, the virus or conjugates of the invention may be delivered by other suitable means, including site-directed injection of the virus bearing the CFTR gene. In the case of CFTR gene delivery, preferred solutions for bronchial instillation are sterile saline solutions containing in the range of from about 1×10^7 to 1×10^{10} pfu/ml, more particularly, in the range of from about 1×10^8 to 1×10^9 pfu/ml of the virus of the present invention.

Other suitable methods for the treatment of cystic fibrosis by use of gene therapy recombinant viruses of this invention may be obtained from the art discussions of other types of gene therapy vectors for CF. See, for example, U. S. Patent No. 5,240,846, incorporated by reference herein.

Example 6 - Synthesis of Polycation Helper Virus Conjugate

Another version of the helper virus of this invention is a polylysine conjugate which enables the pAdΔ shuttle plasmid to complex directly with the helper virus capsid. This conjugate permits efficient delivery of shuttle plasmid pAdΔ shuttle vector in tandem with the helper virus, thereby removing the need for a separate transfection step. See, Fig. 10 for a diagrammatic outline of this construction. Alternatively, such a conjugate with a plasmid supplying some Ad genes and the help r supplying the remaining n cessary g n s for production of the AdΔ viral vector provid s a novel way

to reduce contamination of the helper virus, as discussed above.

Purified stocks of a large-scale expansion of Ad.CBhpAP were modified by coupling poly-L-lysine to the virion capsid essentially as described by K. J. Fisher and J. M. Wilson, Biochem. J., 299:49-58 (1994), resulting in an Ad.CBhpAP-$(Lys)_n$ conjugate. The procedure involves three steps.

First, CsCl band purified helper virus Ad.CBhpAP was reacted with the heterobifunctional crosslinker sulfo-SMCC [sulfo-(N-succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate] (Pierce). The conjugation reaction, which contained 0.5 mg (375 nmol) of sulpho-SMCC and 6 x 10^{12} A_{260} helper virus particles in 3.0 ml of HBS, was incubated at 30°C for 45 minutes with constant gentle shaking. This step involved formation of a peptide bond between the active N-hydroxysuccinimide (NHS) ester of sulpho-SMCC and a free amine (e.g. lysine) contributed by an adenovirus protein sequence (capsid protein) in the vector, yielding a maleimide-activated viral particle. The activated adenovirus is shown in Fig. 10 having the capsid protein fiber labeled with the nucleophilic maleimide moiety. In practice, other capsid polypeptides including hexon and penton base are also targeted.

Unincorporated, unreacted cross-linker was removed by gel filtration on a 1 cm x 15 cm Bio-Gel P-6DG (Bio-Rad Laboratories) column equilibrated with 50 mM Tris/HCl buffer, pH 7.0, and 150 mM NaCl. Peak A_{260} fractions containing maleimide-activated helper virus were combined and placed on ice.

Second, poly-L-lysine having a molecular mass of 58 kDa at 10 mg/ml in 50 mM triethanolamine buffer (pH 8.0), 150 mM NaCl and 1 mM EDTA was thiolated with 2-imminothiolane/HCl (Traut's Reagent; Pierce) to a molar

ratio of 2 moles -SH/mole polylysine under N_2; the cyclic thioimidate reacts with the poly(L-lysine) primary amines resulting in a thiolated polycation. After a 45 minute incubation at room temperature the reaction was applied to a 1 cm x 15 cm Bio-Gel P6DG column equilibrated with 50 mM Tris/HCl buffer (pH 7.0), 150 mM NaCl and 2 mM EDTA to remove unincorporated Traut's Reagent.

Quantification of free thiol groups was accomplished with Ellman's reagent [5,5'-dithio-bis-(2-nitrobenzoic acid)], revealing approximately 3-4 mol of -SH/mol of poly(L-lysine). The coupling reaction was initiated by adding 1 x 10^{12} A_{260} particles of maleimide-activated helper virus/mg of thiolated poly(L-lysine) and incubating the mixture on ice at 4°C for 15 hours under argon. 2-mercaptoethylamine was added at the completion of the reaction and incubation carried out at room temperature for 20 minutes to block unreacted maleimide sites.

Virus-polylysine conjugates, Ad.CPAP-p(Lys)$_n$, were purified away from unconjugated poly(L-lysine) by ultracentrifugation through a CsCl step gradient with an initial composition of equal volumes of 1.45 g/ml (bottom step) and 1.2 g/ml (top step) CsCl in 10 mM Tris/HCl buffer (pH 8.0). Centrifugation was at 90,000 g for 2 hours at 5°C. The final product was dialyzed against 20 mM Hepes buffer (pH 7.8) containing 150 mM NaCl (HBS).

<u>Example 7 - Formation of AdΔ/helper-pLys Viral Particle</u>

The formation of Ad.CBhpAP-pLys/pAdΔ.CMVLacZ particle is initiated by adding 20 μg plasmid pAdΔ.CMVLacZ DNAs to 1.2 x 10^{12} A_{260} particles Ad.CBhpAP-pLys in a final volum of 0.2 ml DMEM and allowing the compl x to d velop at room temp rature for betw en 10-15 minutes. This ratio typically r presents the plasmid DNA binding capacity of a standard lot of ad novirus-pLys

conjugate and gives the highest levels of plasmid transgene expression.

The resulting trans-infection particle is transfected onto 293 cells (4×10^7 cells seeded on a 150 mm dish). Thirty hours after transfection, the particles are recovered and subjected to a freeze/thaw technique to obtain an extract. The extract is purified on a CsCl step gradient with gradients at 1.20 g/ml, 1.36 g/ml and 1.45 g/ml. After centrifugation at 90,000 x g for 8 hours, the AdΔ vectors were obtained from a fraction under the top components as identified by the presence of LacZ, and the helper virus was obtained from a smaller, denser fraction, as identified by the presence of hpAP.

Example 8 - Construction of Modified Helper Viruses with Crippled Packaging (PAC) Sequences

This example refers to Figs. 9A through 9C, 10A and 10B in the design of modified helper viruses of this invention.

Ad5 5' terminal sequences that contained PAC domains I and II (Fig. 8A) or PAC domains I, II, III, and IV (Fig. 8B) were generated by PCR from the wild type Ad5 5' genome depicted in Fig. 1B using PCR clones indicated by the arrows in Fig. 1B. The resulting amplification products (Fig. 8A and 8B) sequences differed from the wild-type Ad5 genome in the number of A-repeats carried by the left (5') end.

As depicted in Fig. 8C, these amplification products were subcloned into the multiple cloning site of pAd.Link.1 (IHGT Vector Core). pAd.Link.1 is a adenovirus based plasmid containing adenovirus m.u. 9.6 through 16.1. The insertion of the modified PAC regions into pAd.Link.1 g nerated two vectors pAd.PACII (containing PAC domains I and II) and pAd.PACIV (containing PAC domains I, II, III, and IV).

Thereafter, as depicted in Figs. 10A and 10B, for each of these plasmids, a human placenta alkaline phosphatase reporter minigene containing the immediate early CMV enhancer/promoter (CMV), human placenta alkaline phosphatase cDNA (hpAP), and SV40 polyadenylation signal (pA), was subcloned into each PAC vector, generating pAd.PACII.CMVhpAP and pAd.PACIV.CMVhpAP, respectively.

These plasmids were then used as substrates for homologous recombination with dl7001 virus, described above, by co-transfection into 293 cells. Homologous recombination occurred between the adenovirus map units 9-16 of the plasmid and the crippled Ad5 virus. The results of homologous recombination were helper viruses containing Ad5 5' terminal sequences that contained PAC domains I and II or PAC domains I, II, III, and IV, followed by the minigene, and Ad5 3' sequences 9.6-78.3 and 87-100. Thus, these crippled viruses are deleted of the E1 gene and the E3 gene.

The plaque formation characteristics of the PAC helper viruses gave an immediate indication that the PAC modifications diminished the rate and extent of growth. Specifically, PAC helper virus plaques did not develop until day 14-21 post-transfection, and on maturation remained small. From previous experience, a standard first generation Ad.CBhpAP helper virus with a complete left terminal sequence would begin to develop by day 7 and mature by day 10.

Viral plaques were picked and suspended in 0.5 ml of DMEM media. A small aliquot of the virus stock was used to infect a fresh monolayer of 293 cells and histochemically stained for recombinant alkaline phosphatas activity 24 hours post-inf ction. Six of eight Ad.PACIV.CMVhpAP (encodes A-rep ats I-IV) clones that wer scre ned for transgene expr ssion w re

positive, while all three Ad.PACII.CMVhpAP clones that were selected scored positive. The clones have been taken through two rounds of plaque purification and are currently being expanded to generate a working stock.

These crippled helper viruses are useful in the production of the AdΔ virus particles according to the procedures described in Example 3. They are characterized by containing sufficient adenovirus genes to permit the packaging of the shuttle vector genome, but their crippled PAC sequences reduce their efficiency for self-encapsidation. Thus less helper viruses are produced in favor of more AdΔ recombinant viruses. Purification of AdΔ virus particles from helper viruses is facilitated in the CsCl gradient, which is based on the weight of the respective viral particles. This facility in purification is a decided advantage of the AdΔ vectors of this invention in contrast to adenovirus vectors having only E1 or smaller deletions. The AdΔ vectors even with minigenes of up to about 15 kb are significantly different in weight than wild type or other adenovirus helpers containing many adenovirus genes.

Example 9 - AdΔ Vector Containing a full-length dystrophin transgene

Duchenne muscular dystrophy (DMD) is a common x-linked genetic disease caused by the absence of dystrophin, a 427K protein encoded by a 14 kilobase transcript. Lack of this important sarcolemmal protein leads to progressive muscle wasting, weakness, and death. One current approach for treating this lethal disease is to transfer a functional copy of the dystrophin gene into the affected muscles. For skeletal muscle, a replication-defective adenovirus repr sents an effici nt delivery system.

According to the present invention, a recombinant plasmid pAdΔ.CMVmdys was created which contains only th Ad5 cis-elements (i.e., ITRs and contiguous packaging sequences) and harbors the full-length murine dystrophin gene driven by the CMV promote.̣. This plasmid was generated as follows.

pSL1180 [Pharmacia Biotech] was cut with *Not* I, filled in by Klenow, and religated thus ablating the *Not* I site in the plasmid. The resulting plasmid is termed pSL1180NN and carries a bacterial ori and *Amp* resistance gene.

pAdΔ.CMVLacZ of Example 1 was cut with EcoRI, klenowed, and ligated with the ApaI-cut pSL1180NN to form pAdΔ.CMV*LacZ* (ApaI).

The 14 kb mouse dystrophin cDNA [sequences provided in C. C. Lee et al, <u>Nature</u>, <u>349</u>:334-336 (1991)] was cloned in two large fragments using a lambda ZAP cloning vector (Stratagene) and subsequently cloned into the bluescript vector pSK- giving rise to the plasmid pCCL-DMD. A schematic diagram of this vector is provided in Fig. 11, which illustrates the restriction enzyme sites.

pAdΔ.CMV*LacZ* (ApaI) was cut with *Not*I and the large fragment gel isolated away from the lacZ cDNA. pCCL-DMD was also cut with *Not*I, gel isolated and subseqently ligated to the large NotI fragment of NotI digested pAdΔ.CMVLacZ (ApaI). The sequences of resulting vector, pAdΔ.CMVmdys, are provided in Fig. 12A-12P [SEQ ID NO:10].

This plasmid contains sequences form the left-end of the Ad5 encompassing bp 1-360 (5' ITR), a mouse dystrophin minigene und r the control of the CMV promoter, and s qu nce from th right end of Ad5 spanning

bp 35353 to the end of the genome (3' ITR). The minigene is followed by an SV-40 poly-A sequence similar to that described for the plasmids described above.

The vector production system described herein is employed. Ten 150mm 293 plat.s are infected at about 90% confluency with a reporter recombinant E1-deleted virus Ad.CBhpAP at an MOI of 5 for 60 minutes at 37°C. These cells are transfected with pAdΔ.CMVmDys by calcium phosphate co-precipitation using 50 μg linearized DNA/dish for about 12-16 hours at 37°C. Media is replaced with DMEM + 10% fetal bovine serum.

Full cytopathic effect is observed and a cell lysate is made by subjecting the cell pellet to freeze-thaw procedures three times. The cells are subjected to an SW41 three tier CsCl gradient for 2 hours and a band migrating between the helper adenovirus and incomplete virus is detected.

Fractions are assayed on a 6 well plate containing 293 cells infected with 5λ of fraction for 16-20 hours in DMEM + 2% FBS. Cells are collected, washed with phosphate buffered saline, and resuspended in 2 ml PBS. 200λ of the 2ml cell fractions is cytospun onto a slide.

The cells were subjected to immunofluorescence for dystrophin as follows. Cells were fixed in 10N MeOH at -20°C. The cells were exposed to a monoclonal antibody specific for the carboxy terminus of human dystrophin [NCL-DYS2; Novocastra Laboratories Ltd., UK]. Cells were then washed three times and exposed to a secondary antibody, i.e. 1:200 goat anti-mouse IgG in FITC.

The titer/fraction for seven fractions revealed in the immunofluorescent stains were calculated by the following formula and reported in Table 2 below.

DFU/field = (DFU/200λ cells) x 10 = DFU/10^6 cells = (DFU/5λ viral fraction) x 20 = DFU/100λ fraction.

Table 2

Fraction	DFU/100λ
1	--
2	--
3	6×10^3
4	1.8×10^4
5	9.6×10^3
6	200
7	200

A virus capable of transducing the dystrophin minigene is detected as a "positive" (i.e., green fluorescent) cell. The results of the IF illustrate that heat-treated fractions do not show positive immunofluorescence. Southern blot data suggest one species on the same size as the input DNA, with helper virus contamination.

The recombinant virus can be subsequently separated from the majority of helper virus by sedimentation through cesium gradients. Initial studies demonstrate that the functional AdCMVΔmDys virions are produced, but are contaminated with helper virus. Successful purification would render AdΔ virions that are incapable of encoding viral proteins but are capable of transducing murine skeletal muscle.

Example 10 - Pseudotyping

The following experiment provides a method for preparing a recombinant AdΔ according to the invention, utilizing helper viruses from ser types which differ from that of the pAdΔ in the transfection/infection protocol. It is unexpected that the ITRs and packaging sequence of

Ad5 could be incorporated into a virion of another serotype.

A. Protocol

The basic approach is to transfect the AdΔ.CMV*lacZ* recombinant virus (Ad5) into 293 cells and subsequently infect the cell with the helper virus derived from a variety of Ad serotypes (2, 3, 4, 5, 7, 8, 12, and 40). When CPE is achieved, the lysate is harvested and banded through two cesium gradients.

More particularly, the Ad5-based plasmid pAdΔ.CMV*lacZ* of Example 1 was linearized with EcoRI. The linearized plasmids were then transfected into ten 150 mm dishes of 293 cells using calcium phosphate co-precipitation. At 10-15 hours post transfection, wild type adenoviruses (of one of the following serotypes: 2, 3, 4, 5, 7, 12, 40) were used to infect cells at an MOI of 5. The cells were then harvested at full CPE and lysed by three rounds of freeze-thawing. Pellet is resuspended in 4 mL Tris-HCl. Cell debris was removed by centrifugation and partial purification of Ad5Δ.CMV*lacZ* from helper virus was achieved with 2 rounds of CsCl gradient centrifugation (SW41 column, 35,000 rpm, 2 hours). Fractions were collected from the bottom of the tube (fraction #1) and analysed for *lacZ* transducing viruses on 293 target cells by histochemical staining (at 20h PI). Contaminating helper viruses were quantitated by plaque assay.

Except for adenovirus type 3, infection with Ad serotypes 2, 4, 5, 7, 12 and 40 were able to produce *lacZ* transducing viruses. The peak of β-galactosidase activity was detected between the two major A_{260} absorbing peaks, where most of the helper viruses banded (data not shown). The quantity of *lacZ* virus recovered from 10 plates ranged from 10^4 to 10^8 transducing particles depending on the serotype of the helper. As

expected Ad2 and Ad5 produced the highest titer of *lacZ* transducing viruses (Table 3). Wild type contamination was in general 10^2-10^3 log higher than corresponding *lacZ* titer except in the case of Ad40.

B. Results

Table 3 summarizes the growth characteristics of the wild type adenoviruses as evaluated on propagation in 293 cells. This demonstrated the feasibility of utilizing these helper viruses to infect the cell line which has been transfected with the Ad5 deleted virus.

Table 3

Adenovirus serotypes	p/ml	pfu/ml	p:pfu
2	5×10^{12}	2.5×10^{11}	20:01
3	1×10^{12}	6.25×10^{9}	160:1
4	3×10^{12}	2×10^{9}	150:1
5	1×10^{12}	5×10^{10}	20:01
7a	5×10^{12}	1×10^{11}	50:1
12	6×10^{11}	4×10^{9}	150:1
35	1.2×10^{12}		
40	2.2×10^{12}	4.4×10^{8}	5000:1

Table 4 summarizes the results of the final purified fractions. The middle column, labeled LFU/μl quantifies the production of *lacZ* forming units, which is a direct measure of the packaging and propagation of pseudotyped recombinant AdΔ virus. The pfu/μl titer is an estimate of the contaminating wild type virus. AdΔ virus pseudotyped with all adenoviral strains was generated except for Ad3. The titers range between 10^7 - 10^4.

Table 4

Serotypes	LFU/ml	PFU/ml
2	4.6×10^7	1.8×10^9
3	0	NA
4	6.7×10^6	9.3×10^7
5	6.3×10^7	1.9×10^9
7a	3×10^6	1.8×10^8
12	1.2×10^5	3.3×10^8
40	9.5×10^4	1.5×10^3

Table 5A-5D represents a more detailed analysis of the fractions from the second purification for each of the experiments summarized in Table 4. Again, LFU/μl is the recovery of the AdΔ viruses, whereas pfu/μl represents recovery of the helper virus.

Table 5A

Ad2 Fraction #	VOLUME/ul	LFU/ul	PFU/ul
1	120	9532	8×10^6
2	100	5.8×10^4	3×10^6
3	100	8.24×10^4	6×10^5
4	100	9.47×10^4	1.2×10^5
5	100	6×10^4	8×10^4
6	100	2×10^4	6×10^4
7	100	5434	5×10^4
Total/10 pH		3.32×10^7	1.35×10^9

Table 5B

Ad4 Fraction #	VOLUME/ul	LFU/ul	PFU/ul
1	100	1000	1.75×10^5
2	100	1.79×10^4	2.8×10^5
3	100	1.8×10^4	5.5×10^4
4	100	2909	1.25×10^4
5	100	920	4×10^4
6	100	153	3×10^3
Total/10 pH		4×10^6	5.6×10^7
Ad5 Fraction #			
1	120	1.98×10^4	6×10^6
2	100	5.8×10^4	3×10^6
3	100	1.2×10^5	1.5×10^6
4	100	1×10^5	1.4×10^5
5	100	7.96×10^4	8×10^4
6	100	6860	6×10^4
Total/10 pH		3.88×10^7	1.2×10^9

Table 5C

Ad7 Fraction #	VOLUME/ul	LFU/ul	PFU/ul
1	100	1225	5×10^5
2	100	5550	4×10^5
3	100	4938	2×10^5
4	100	3866	8×10^4
5	100	4134	6×10^4
6	100	995	7×10^4
7	100	230	6×10^3
Total/10 pH		2.09×10^6	1.3×10^8
Ad12 Fraction #			
1	100	31	5×10^5
2	80	169	8.5×10^5
3	80	245	1.8×10^5
4	110	161	1.1×10^5
5	120	62	7×10^3
Total/10 pH		6.14×10^4	1.65×10^8

Table 5D

Ad40 Fraction #	VOLUME/ul	LFU/ul	PFU/ul
1	80	61	5
2	80	184	3
3	80	199	3
4	80	168	1
5	80	122	
6	100	46	
7	100	32	
Total/10 pH		6.65×10^4	1.1×10^3

C. <u>Characterization of the Structure of Packaged Viruses</u>

Aliquots of serial fractions were analysed by Southern blots using *lacZ* as a probe. In the case of Ad2 and 5, not only the linearized monomer was packaged but multiple forms of recombinant virus with distinct sizes were found. These forms correlated well with the sizes of dimers, trimers and other higher molecular weight concatamers. The linearized monomers peaked closer to the top of tube (the defective adenovirus band) than other forms. When these forms were correlated with *lacZ* activity, a better correlation was found between the higher molecular weight forms than the monomers. With pseudotyping of Ad4 and Ad7, no linearized monomers were packaged and only higher molecular weight forms were found.

These data definitiv ly d monstrate the production and characterization of the Δ virus and the different pseudotypes. This example illustrates a very simple way of generating pseudotype viruses.

Example 11 - AdΔ Vector Containing a FH Gene

Familial hypercholesterolemia (FH) is an autosomal dominant disorder caused by abnormalities (deficiencies) in the function or expression of LDL receptors [M.S. Brown and J.L. Goldstein, Science, 232(4746):34-37 (1986); J.L. Goldstein and M.S. Brown, "Familial hypercholesterolemia" in Metabolic Basis of Inherited Disease., ed. C.R. Scriver et al, McGraw Hill, New York, pp1215-1250 (1989).] Patients who inherit one abnormal allele have moderate elevations in plasma LDL and suffer premature life-threatening coronary artery disease (CAD). Homozygous patients have severe hypercholesterolemia and life-threatening CAD in childhood. An FH-containing vector of the invention is constructed by replacing the lacZ minigene in the pAdΔc.CMVlacZ vector with a minigene containing the LDL receptor gene [T. Yamamoto et al, Cell, 39:27-38 (1984)] using known techniques and as described analogously for the dystrophin gene and CFTR in the preceding examples. Vectors bearing the LDL receptor gene can be readily constructed according to this invention. The resulting plasmid is termed pAdΔc.CMV-LDL.

This plasmid is useful in gene therapy of FH alone, or preferably, in the form of a conjugate prepared as described herein to substitute a normal LDL gene for the abnormal allele responsible for the gene.

A. Ex Vivo Gene Therapy

Ex vivo gene therapy can be performed by harvesting and establishing a primary culture of hepatocytes from a patient. Known techniques may be used to isolate and transduce the hepatocytes with the above vector(s) bearing the LDL receptor gene(s). For example, techniques of collag nase perfusion developed for rabbit liver can be adapt d for human tissue and us d in transduction. Following transduction, the hepatocytes

are removed from the tissue culture plates and reinfused into the patient using known techniques, e.g. via a catheter placed into the inferior mesenteric vein.

B. In Vivo Gene Therapy

Desirably, the *in vivo* approach to gene therapy, e.g. liver-directed, involves the use of the vectors and vector conjugates described above. A preferred treatment involves infusing a vector LDL conjugate of this invention into the peripheral circulation of the patient. The patient is then evaluated for change in serum lipids and liver tissues.

The virus or conjugate can be used to infect hepatocytes *in vivo* by direct injection into a peripheral or portal vein (10^7-10^8 pfu/kg) or retrograde into the biliary tract (same dose). This effects gene transfer into the majority of hepatocytes.

Treatments are repeated as necessary, e.g. weekly. Administration of a dose of virus equivalent to an MOI of approximately 20 (i.e. 20 pfu/hepatocyte) is anticipated to lead to high level gene expression in the majority of hepatocytes.

All references recited above are incorporated herein by reference. Numerous modifications and variations of the present invention are included in the above-identified specification and are expected to be obvious to one of skill in the art. Such modifications and alternations to the compositions and processes of the present invention, such as various modifications to the PAC sequences or the shuttle vectors, or to other sequences of the vector, helper virus and minigene components, are believed to be encompassed in the scope of the claims appended hereto.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Trustees of the University of Pennsylvania
Wilson, James M.
Fisher, Krishna J.
Chen, Shu-Jen
Weitzman, Matthew

(ii) TITLE OF INVENTION: Improved Adenovirus and Methods of Use Thereof

(iii) NUMBER OF SEQUENCES: 10

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Howson and Howson
(B) STREET: Spring House Corporate Cntr, PO Box 457
(C) CITY: Spring House
(D) STATE: Pennsylvania
(E) COUNTRY: USA
(F) ZIP: 19477

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/331,381
(B) FILING DATE: 28-OCT-1994

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Bak, Mary E.
(B) REGISTRATION NUMBER: 31,215
(C) REFERENCE/DOCKET NUMBER: GNVPN.008PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 215-540-9200
(B) TELEFAX: 215-540-5818

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7897 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GAACTCGAGC AGCTGAAGCT TGAATTCCAT CATCAATAAT ATACCTTATT 50

TTGGATTGAA GCCAATATGA TAATGAGGGG GTGGAGTTTG TGACGTGGCG 100

CGGGGCGTGG GAACGGGGCG GGTGACGTAG GTTTTAGGGC GGAGTAACTT 150

GTATGTGTTG GGAATTGTAG TTTTCTTAAA ATGGGAAGTT ACGTAACGTG 200

GGAAAACGGA AGTGACGATT TGAGGAAGTT GTGGGTTTTT TGGCTTTCGT 250

TTCTGGGCGT AGGTTCGCGT GCGGTTTTCT GGGTGTTTTT TGTGGACTTT 300

AACCGTTACG TCATTTTTTA GTCCTATATA TACTCGCTCT GCACTTGGCC 350

CTTTTTTACA CTGTGACTGA TTGAGCTGGT GCCGTGTCGA GTGGTGTTTT 400

TTTAATAGGT TTTCTTTTTT ACTGGTAAGG CTGACTGTTA GGCTGCCGCT 450

GTGAAGCGCT GTATGTTGTT CTGGAGCGGG AGGGTGCTAT TTTGCCTAGG 500

CAGGAGGGTT TTTCAGGTGT TTATGTGTTT TTCTCTCCTA TTAATTTTGT 550

TATACCTCCT ATGGGGGCTG TAATGTTGTC TCTACGCCTG CGGGTATGTA 600

TTCCCCCCAA GCTTGCATGC CTGCAGGTCG ACTCTAGAGG ATCCGAAAAA 650

ACCTCCCACA CCTCCCCCTG AACCTGAAAC ATAAAATGAA TGCAATTGTT 700

GTTGTTAACT TGTTTATTGC AGCTTATAAT GGTTACAAAT AAAGCAATAG 750

CATCACAAAT TTCACAAATA AAGCATTTTT TTCACTGCAT TCTAGTTGTG 800

GTTTGTCCAA ACTCATCAAT GTATCTTATC ATGTCTGGAT CCCCGCGGCC 850

GCCTAGAGTC GAGGCCGAGT TTGTCAGAAA GCAGACCAAA CAGCGGTTGG 900

AATAATAGCG AGAACAGAGA AATAGCGGCA AAAATAATAC CCGTATCACT 950

TTTGCTGATA TGGTTGATGT CATGTAGCCA AATCGGGAAA AACGGGAAGT 1000

AGGCTCCCAT GATAAAAAAG TAAAAGAAAA AGAATAAACC GAACATCCAA 1050

AAGTTTGTGT TTTTTAAATA GTACATAATG GATTTCCTTA CGCGAAATAC 1100

GGGCAGACAT GGCCTGCCCG GTTATTATTA TTTTTGACAC CAGACCAACT 1150

GGTAATGGTA GCGACCGGCG CTCAGCTGTA A.TCCGCCGA TACTGACGGG 1200

CTCCAGGAGT CGTCGCCACC AATCCCCATA TGGAAACCGT CGATATTCAG 1250

CCATGTGCCT TCTTCCGCGT GCAGCAGATG GCGATGGCTG CTTTCCATCA 1300

GTTGCTGTTG ACTGTAGCGG CTGATGTTGA ACTGGAAGTC GCCGCGCCAC 1350

TGGTGTGGGC CATAATTCAA TTCGCGCGTC CCGCAGCGCA GACCGTTTTC 1400

GCTCGGGAAG ACGTACGGGG TATACATGTC TGACAATGGC AGATCCCAGC 1450

GGTCAAAACA GGCGGCAGTA AGGCGGTCGG GATAGTTTTC TTGCGGCCCT 1500

AATCCGAGCC AGTTTACCCG CTCTGCTACC TGCGCCAGCT GGCAGTTCAG 1550

GCCAATCCGC GCCGGATGCG GTGTATCGCT CGCCACTTCA ACATCAACGG 1600

TAATCGCCAT TTGACCACTA CCATCAATCC GGTAGGTTTT CCGGCTGATA 1650

AATAAGGTTT TCCCCTGATG CTGCCACGCG TGAGCGGTCG TAATCAGCAC 1700

CGCATCAGCA AGTGTATCTG CCGTGCACTG CAACAACGCT GCTTCGGCCT 1750

GGTAATGGCC CGCCGCCTTC CAGCGTTCGA CCCAGGCGTT AGGGTCAATG 1800

CGGGTCGCTT CACTTACGCC AATGTCGTTA TCCAGCGGTG CACGGGTGAA 1850

CTGATCGCGC AGCGGCGTCA GCAGTTGTTT TTTATCGCCA ATCCACATCT 1900

GTGAAAGAAA GCCTGACTGG CGGTTAAATT GCCAACGCTT ATTACCCAGC 1950

TCGATGCAAA AATCCATTTC GCTGGTGGTC AGATGCGGGA TGGCGTGGGA 2000

CGCGGCGGGG AGCGTCACAC TGAGGTTTTC CGCCAGACGC CACTGCTGCC 2050

AGGCGCTGAT GTGCCCGGCT TCTGACCATG CGGTCGCGTT CGGTTGCACT 2100

ACGCGTACTG TGAGCCAGAG TTGCCCGGCG CTCTCCGGCT GCGGTAGTTC 2150

AGGCAGTTCA ATCAACTGTT TACCTTGTGG AGCGACATCC AGAGGCACTT 2200

CACCGCTTGC CAGCGGCTTA CCATCCAGCG CCACCATCCA GTGCAGGAGC 2250

TCGTTATCGC TATGACGGAA CAGGTATTCG CTGGTCACTT CGATGGTTTG 2300

CCCGGATAAA CGGAACTGGA AAAACTGCTG CTGGTGTTTT GCTTCCGTCA 2350

GCGCTGGATG CGGCGTGCGG TCGGCAAAGA CCAGACCGTT CATACAGAAC 2400

TGGCGATCGT TCGGCGTATC GCCAAAATCA CCGCCGTAAG CCGACCACGG 2450

GTTGCCGTTT TCATCATATT TAATCAGCGA CT[illegible]ATCCACC CAGTCCCAGA 2500

CGAAGCCGCC CTGTAAACGG GGATACTGAC GAAACGCCTG CCAGTATTTA 2550

GCGAAACCGC CAAGACTGTT ACCCATCGCG TGGGCGTATT CGCAAAGGAT 2600

CAGCGGGCGC GTCTCTCCAG GTAGCGAAAG CCATTTTTTG ATGGACCATT 2650

TCGGCACAGC CGGGAAGGGC TGGTCTTCAT CCACGCGCGC GTACATCGGG 2700

CAAATAATAT CGGTGGCCGT GGTGTCGGCT CCGCCGCCTT CATACTGCAC 2750

CGGGCGGGAA GGATCGACAG ATTTGATCCA GCGATACAGC GCGTCGTGAT 2800

TAGCGCCGTG GCCTGATTCA TTCCCCAGCG ACCAGATGAT CACACTCGGG 2850

TGATTACGAT CGCGCTGCAC CATTCGCGTT ACGCGTTCGC TCATCGCCGG 2900

TAGCCAGCGC GGATCATCGG TCAGACGATT CATTGGCACC ATGCCGTGGG 2950

TTTCAATATT GGCTTCATCC ACCACATACA GGCCGTAGCG GTCGCACAGC 3000

GTGTACCACA GCGGATGGTT CGGATAATGC GAACAGCGCA CGGCGTTAAA 3050

GTTGTTCTGC TTCATCAGCA GGATATCCTG CACCATCGTC TGCTCATCCA 3100

TGACCTGACC ATGCAGAGGA TGATGCTCGT GACGGTTAAC GCCTCGAATC 3150

AGCAACGGCT TGCCGTTCAG CAGCAGCAGA CCATTTTCAA TCCGCACCTC 3200

GCGGAAACCG ACATCGCAGG CTTCTGCTTC AATCAGCGTG CCGTCGGCGG 3250

TGTGCAGTTC AACCACCGCA CGATAGAGAT TCGGGATTTC GGCGCTCCAC 3300

AGTTTCGGGT TTTCGACGTT CAGACGTAGT GTGACGCGAT CGGCATAACC 3350

ACCACGCTCA TCGATAATTT CACCGCCGAA AGGCGCGGTG CCGCTGGCGA 3400

CCTGCGTTTC ACCCTGCCAT AAAGAAACTG TTACCCGTAG GTAGTCACGC 3450

AACTCGCCGC ACATCTGAAC TTCAGCCTCC AGTACAGCGC GGCTGAAATC 3500

ATCATTAAAG CGAGTGGCAA CATGGAAATC GCTGATTTGT GTAGTCGGTT 3550

TATGCAGCAA CGAGACGTCA CGGAAAATGC CGCTCATCCG CCACATATCC 3600

TGATCTTCCA GATAACTGCC GTCACTCCAA CGCAGCACCA TCACCGCGAG 3650

GCGGTTTTCT CCGGCGCGTA AAAATGCGCT CAGGTCAAAT TCAGACGGCA 3700

AACGACTGTC CTGGCCGTAA CCGACCCAGC GCCCGTTGCA CCACAGATGA 3750

AACGCCGAGT TAACGCCATC AAAAATAATT CGCGTCTGGC CTTCCTGTAG 3800

CCAGCTTTCA TCAACATTAA ATGTGAGCGA GTAACAACCC GTCGGATTCT 3850

CCGTGGGAAC AAACGGCGGA TTGACCGTAA TGGGATAGGT TACGTTGGTG 3900

TAGATGGGCG CATCGTAACC GTGCATCTGC CAGTTTGAGG GGACGACGAC 3950

AGTATCGGCC TCAGGAAGAT CGCACTCCAG CCAGCTTTCC GGCACCGCTT 4000

CTGGTGCCGG AAACCAGGCA AAGCGCCATT CGCCATTCAG GCTGCGCAAC 4050

TGTTGGGAAG GGCGATCGGT GCGGGCCTCT TCGCTATTAC GCCAGCTGGC 4100

CAAAGGGGGA TGTGCTGCAA GGCGATTAAG TTGGGTAACG CCAGGGTTTT 4150

CCCAGTCACG ACGTTGTAAA ACGACGGGAT CGCGCTTGAG CAGCTCCTTG 4200

CTGGTGTCCA GACCAATGCC TCCCAGACCG GCAACGAAAA TCACGTTCTT 4250

GTTGGTCAAA GTAAACGACA TGGTGACTTC TTTTTTGCTT TAGCAGGCTC 4300

TTTCGATCCC CGGGAATTGC GGCCGCGGGT ACAATTCCGC AGCTTTTAGA 4350

GCAGAAGTAA CACTTCCGTA CAGGCCTAGA AGTAAAGGCA ACATCCACTG 4400

AGGAGCAGTT CTTTGATTTG CACCACCACC GGATCCGGGA CCTGAAATAA 4450

AAGACAAAAA GACTAAACTT ACCAGTTAAC TTTCTGGTTT TTCAGTTCCT 4500

CGAGTACCGG ATCCTCTAGA GTCCGGAGGC TGGATCGGTC CCGGTCTCTT 4550

CTATGGAGGT CAAAACAGCG TGGATGGCGT CTCCAGGCGA TCTGACGGTT 4600

CACTAAACGA GCTCTGCTTA TATAGACCTC CCACCGTACA CGCCTACCGC 4650

CCATTTGCGT CAATGGGGCG GAGTTGTTAC GACATTTTGG AAAGTCCCGT 4700

TGATTTTGGT GCCAAAACAA ACTCCCATTG ACGTCAATGG GGTGGAGACT 4750

TGGAAATCCC CGTGAGTCAA ACCGCTATCC ACGCCCATTG ATGTACTGCC 4800

AAAACCGCAT CACCATGGTA ATAGCGATGA CTAATACGTA GATGTACTGC 4850

CAAGTAGGAA AGTCCCATAA GGTCATGTAC TGGGCATAAT GCCAGGCGGG 4900

CCATTTACCG TCATTGACGT CAATAGGGGG CGTACTTGGC ATATGATACA 4950

CTTGATGTAC TGCCAAGTGG GCAGTTTACC GTAAATACTC CACCCATTGA 5000

CGTCAATGGA AAGTCCCTAT TGGCGTTACT ATGGGAACAT ACGTCATTAT 5050

TGACGTCAAT GGGCGGGGGT CGTTGGGCGG TC[illegible]GCCAGGC GGGCCATTTA 5100

CCGTAAGTTA TGTAACGACC TGCAGGTCGA CTCTAGAGGA TCTCCCTAGA 5150

CAAATATTAC GCGCTATGAG TAACACAAAA TTATTCAGAT TTCACTTCCT 5200

CTTATTCAGT TTTCCCGCGA AAATGGCCAA ATCTTACTCG GTTACGCCCA 5250

AATTTACTAC AACATCCGCC TAAAACCGCG CGAAAATTGT CACTTCCTGT 5300

GTACACCGGC GCACACCAAA AACGTCACTT TTGCCACATC CGTCGCTTAC 5350

ATGTGTTCCG CCACACTTGC AACATCACAC TTCCGCCACA CTACTACGTC 5400

ACCCGCCCCG TTCCCACGCC CCGCGCCACG TCACAAACTC CACCCCCTCA 5450

TTATCATATT GGCTTCAATC CAAAATAAGG TATATTATTG ATGATGCTAG 5500

CGAATTCATC GATGATATCA GATCTGCCGG TCTCCCTATA GTGAGTCGTA 5550

TTAATTTCGA TAAGCCAGGT TAACCTGCAT TAATGAATCG GCCAACGCGC 5600

GGGGAGAGGC GGTTTGCGTA TTGGGCGCTC TTCCGCTTCC TCGCTCACTG 5650

ACTCGCTGCG CTCGGTCGTT CGGCTGCGGC GAGCGGTATC AGCTCACTCA 5700

AAGGCGGTAA TACGGTTATC CACAGAATCA GGGGATAACG CAGGAAAGAA 5750

CATGTGAGCA AAAGGCCAGC AAAAGGCCAG GAACCGTAAA AAGGCCGCGT 5800

TGCTGGCGTT TTTCCATAGG CTCCGCCCCC CTGACGAGCA TCACAAAAAT 5850

CGACGCTCAA GTCAGAGGTG GCGAAACCCG ACAGGACTAT AAAGATACCA 5900

GGCGTTTCCC CCTGGAAGCT CCCTCGTGCG CTCTCCTGTT CCGACCCTGC 5950

CGCTTACCGG ATACCTGTCC GCCTTTCTCC CTTCGGGAAG CGTGGCGCTT 6000

TCTCAATGCT CACGCTGTAG GTATCTCAGT TCGGTGTAGG TCGTTCGCTC 6050

CAAGCTGGGC TGTGTGCACG AACCCCCCGT TCAGCCCGAC CGCTGCGCCT 6100

TATCCGGTAA CTATCGTCTT GAGTCCAACC CGGTAAGACA CGACTTATCG 6150

CCACTGGCAG CAGCCACTGG TAACAGGATT AGCAGAGCGA GGTATGTAGG 6200

CGGTGCTACA GAGTTCTTGA AGTGGTGGCC TAACTACGGC TACACTAGAA 6250

GGACAGTATT TGGTATCTGC GCTCTGCTGA AGCCAGTTAC CTTCGGAAAA 6300

AGAGTTGGTA GCTCTTGATC CGGCAAACAA ACCACCGCTG CTAGCGGTGG 6350

TTTTTTTGTT TGCAAGCAGC AGATTACGCG CAGAAAAAAA GGATCTCAAG 6400

AAGATCCTTT GATCTTTTCT ACGGGGTCTG ACGCTCAGTG GAACGAAAAC 6450

TCACGTTAAG GGATTTTGGT CATGAGATTA TCAAAAAGGA TCTTCACCTA 6500

GATCCTTTTA AATTAAAAAT GAAGTTTTAA ATCAATCTAA AGTATATATG 6550

AGTAAACTTG GTCTGACAGT TACCAATGCT TAATCAGTGA GGCACCTATC 6600

TCAGCGATCT GTCTATTTCG TTCATCCATA GTTGCCTGAC TCCCCGTCGT 6650

GTAGATAACT ACGATACGGG AGGGCTTACC ATCTGGCCCC AGTGCTGCAA 6700

TGATACCGCG AGACCCACGC TCACCGGCTC CAGATTTATC AGCAATAAAC 6750

CAGCCAGCCG GAAGGGCCGA GCGCAGAAGT GGTCCTGCAA CTTTATCCGC 6800

CTCCATCCAG TCTATTAATT GTTGCCGGGA AGCTAGAGTA AGTAGTTCGC 6850

CAGTTAATAG TTTGCGCAAC GTTGTTGCCA TTGCTACAGG CATCGTGGTG 6900

TCACGCTCGT CGTTTGGTAT GGCTTCATTC AGCTCCGGTT CCCAACGATC 6950

AAGGCGAGTT ACATGATCCC CCATGTTGTG CAAAAAAGCG GTTAGCTCCT 7000

TCGGTCCTCC GATCGTTGTC AGAAGTAAGT TGGCCGCAGT GTTATCACTC 7050

ATGGTTATGG CAGCACTGCA TAATTCTCTT ACTGTCATGC CATCCGTAAG 7100

ATGCTTTTCT GTGACTGGTG AGTACTCAAC CAAGTCATTC TGAGAATAGT 7150

GTATGCGGCG ACCGAGTTGC TCTTGCCCGG CGTCAATACG GGATAATACC 7200

GCGCCACATA GCAGAACTTT AAAAGTGCTC ATCATTGGAA AACGTTCTTC 7250

GGGGCGAAAA CTCTCAAGGA TCTTACCGCT GTTGAGATCC AGTTCGATGT 7300

AACCCACTCG TGCACCCAAC TGATCTTCAG CATCTTTTAC TTTCACCAGC 7350

GTTTCTGGGT GAGCAAAAAC AGGAAGGCAA AATGCCGCAA AAAAGGGAAT 7400

AAGGGCGACA CGGAAATGTT GAATACTCAT ACTCTTCCTT TTTCAATATT 7450

ATTGAAGCAT TTATCAGGGT TATTGTCTCA TGAGCGGATA CATATTTGAA 7500

TGTATTTAGA AAAATAAACA AATAGGGGTT CCGCGCACAT TTCCCCGAAA 7550

AGTGCCACCT GACGTCTAAG AAACCATTAT TATCATGACA TTAACCTATA 7600

AAAATAGGCG TATCACGAGG CCCTTTCGTC TCGCGCGTTT CGGTGATGAC 7650

GGTGAAAACC TCTGACACAT GCAGCTCCCG GAGACGGTCA CAGCTTGTCT 7700

GTAAGCGGAT GCCGGGAGCA GACAAGCCCG TCAGGGCGCG TCAGCGGGTG 7750

TTGGCGGGTG TCGGGGCTGG CTTAACTATG CGGCATCAGA GCAGATTGTA 7800

CTGAGAGTGC ACCATATGGA CATATTGTCG TTAGAACGCG GCTACAATTA 7850

ATACATAACC TTATGTATCA TACACATACG ATTTAGGTGA CACTATA 7897

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7852 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GAATTCGCTA GCTAGCGGGG GAATACATAC CCGCAGGCGT AGAGACAACA 50

TTACAGCCCC CATAGGAGGT ATAACAAAAT TAATAGGAGA GAAAAACACA 100

TAAACACCTG AAAAACCCTC CTGCCTAGGC AAAATAGCAC CCTCCCGCTC 150

CAGAACAACA TACAGCGCTT CACAGCGGCA GCCTAACAGT CAGCCTTACC 200

AGTAAAAAAG AAAACCTATT AAAAAAACAC CACTCGACAC GGCACCAGCT 250

CAATCAGTCA CAGTGTAAAA AAGGGCCAAG TGCAGAGCGA GTATATATAG 300

GACTAAAAAA TGACGTAACG GTTAAAGTCC ACAAAAAACA CCCAGAAAAC 350

CGCACGCGAA CCTACGCCCA GAAACGAAAG CCAAAAAACC CACAACTTCC 400

TCAAATCGTC ACTTCCGTTT TCCCACGTTA CGTAACTTCC CATTTTAAGA 450

AAACTACAAT TCCCAACACA TACAAGTTAC TCCGCCCTAA AACCTACGTC 500

ACCCGCCCCG TTCCCACGCC CCGCGCCACG TCACAAACTC CACCCCCTCA 550

TTATCATATT GGCTTCAATC CAAAATAAGG TATATTATTG ATGATGCTAG 600

CATCATCAAT AATATACCTT ATTTTGGATT GAAGCCAATA TGATAATGAG 650

GGGGTGGAGT TTGTGACGTG GCGCGGGGCG TGGGAACGGG GCGGGTGACG 700

TAGTAGTGTG GCGGAAGTGT GATGTTGCAA GTGTGGCGGA ACACATGTAA 750

GCGACGGATG TGGCAAAAGT GACGTTTTTG GTGTGCGCCG GTGTACACAG 800

GAAGTGACAA TTTTCGCGCG GTTTTAGGCG GATGTTGTAG TAAATTTGGG 850

CGTAACCGAG TAAGATTTGG CCATTTTCGC GGGAAAACTG AATAAGAGGA 900

AGTGAAATCT GAATAATTTT GTGTTACTCA TAGCGCGTAA TATTTGTCTA 950

GGGAGATCAG CCTGCAGGTC GTTACATAAC TTACGGTAAA TGGCCCGCCT 1000

GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT 1050

TCCCATAGTA ACGCCAATAG GGACTTTCCA TTGACGTCAA TGGGTGGAGT 1100

ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA 1150

AGTACGCCCC CTATTGACGT CAATGACGGT AAATGGCCCG CCTGGCATTA 1200

TGCCCAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG 1250

TATTAGTCAT CGCTATTACC ATGGTGATGC GGTTTTGGCA GTACATCAAT 1300

GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT 1350

TGACGTCAAT GGGAGTTTGT TTTGGCACCA AAATCAACGG GACTTTCCAA 1400

AATGTCGTAA CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTA 1450

CGGTGGGAGG TCTATATAAG CAGAGCTCGT TTAGTGAACC GTCAGATCGC 1500

CTGGAGACGC CATCCACGCT GTTTTGACCT CCATAGAAGA CACCGGGACC 1550

GATCCAGCCT CCGGACTCTA GAGGATCCGG TACTCGAGGA ACTGAAAAAC 1600

CAGAAAGTTA ACTGGTAAGT TTAGTCTTTT TGTCTTTTAT TTCAGGTCCC 1650

GGATCCGGTG GTGGTGCAAA TCAAAGAACT GCTCCTCAGT GGATGTTGCC 1700

TTTACTTCTA GGCCTGTACG GAAGTGTTAC TTCTGCTCTA AAAGCTGCGG 1750

AATTGTACCC GCGGCCGCAA TTCCCGGGGA TCGAAAGAGC CTGCTAAAGC 1800

AAAAAAGAAG TCACCATGTC GTTTACTTTG ACCAACAAGA ACGTGATTTT 1850

CGTTGCCGGT CTGGGAGGCA TTGGTCTGGA CACCAGCAAG GAGCTGCTCA 1900
AGCGCGATCC CGTCGTTTTA CAACGTCGTG ACTGGGAAAA CCCTGGCGTT 1950
ACCCAACTTA ATCGCCTTGC AGCACATCCC CCTTTCGCCA GCTGGCGTAA 2000
TAGCGAAGAG GCCCGCACCG ATCGCCCTTC CCAACAGTTG CGCAGCCTGA 2050
ATGGCGAATG GCGCTTTGCC TGGTTTCCGG CACCAGAAGC GGTGCCGGAA 2100
AGCTGGCTGG AGTGCGATCT TCCTGAGGCC GATACTGTCG TCGTCCCCTC 2150
AAACTGGCAG ATGCACGGTT ACGATGCGCC CATCTACACC AACGTAACCT 2200
ATCCCATTAC GGTCAATCCG CCGTTTGTTC CCACGGAGAA TCCGACGGGT 2250
TGTTACTCGC TCACATTTAA TGTTGATGAA AGCTGGCTAC AGGAAGGCCA 2300
GACGCGAATT ATTTTTGATG GCGTTAACTC GGCGTTTCAT CTCTGGTGCA 2350
ACGGGCGCTG GGTCGGTTAC GGCCAGGACA GTCGTTTGCC GTCTGAATTT 2400
GACCTGAGCG CATTTTTACG CGCCGGAGAA AACCGCCTCG CGGTGATGGT 2450
GCTGCGTTGG AGTGACGGCA GTTATCTGGA AGATCAGGAT ATGTGGCGGA 2500
TGAGCGGCAT TTTCCGTGAC GTCTCGTTGC TGCATAAACC GACTACACAA 2550
ATCAGCGATT TCCATGTTGC CACTCGCTTT AATGATGATT TCAGCCGCGC 2600
TGTACTGGAG GCTGAAGTTC AGATGTGCGG CGAGTTGCGT GACTACCTAC 2650
GGGTAACAGT TTCTTTATGG CAGGGTGAAA CGCAGGTCGC CAGCGGCACC 2700
GCGCCTTTCG GCGGTGAAAT TATCGATGAG CGTGGTGGTT ATGCCGATCG 2750
CGTCACACTA CGTCTGAACG TCGAAAACCC GAAACTGTGG AGCGCCGAAA 2800
TCCCGAATCT CTATCGTGCG GTGGTTGAAC TGCACACCGC CGACGGCACG 2850
CTGATTGAAG CAGAAGCCTG CGATGTCGGT TTCCGCGAGG TGCGGATTGA 2900
AAATGGTCTG CTGCTGCTGA ACGGCAAGCC GTTGCTGATT CGAGGCGTTA 2950
ACCGTCACGA GCATCATCCT CTGCATGGTC AGGTCATGGA TGAGCAGACC 3000
ATGGTGCAGG ATATCCTGCT GATGAAGCAG AACAACTTTA ACGCCGTGCG 3050
CTGTTCGCAT TATCCGAACC ATCCGCTGTG GTACACGCTG TGCGACCGCT 3100
ACGGCCTGTA TGTGGTGGAT GAAGCCAATA TTGAAACCCA CGGCATGGTG 3150

CCAATGAATC GTCTGACCGA TGATCCGCGC TGGCTACCGG CGATGAGCGA 3200
ACGCGTAACG CGAATGGTGC AGCGCGATCG TAATCACCCG AGTGTGATCA 3250
TCTGCTCGCT GGGGAATGAA TCAGGCCACG GCGCTAATCA CGACGCGCTG 3300
TATCGCTGGA TCAAATCTGT CGATCCTTCC CGCCCGGTGC AGTATGAAGG 3350
CGGCGGAGCC GACACCACGG CCACCGATAT TATTTGCCCG ATGTACGCGC 3400
GCGTGGATGA AGACCAGCCC TTCCCGGCTG TGCCGAAATG GTCCATCAAA 3450
AAATGGCTTT CGCTACCTGG AGAGACGCGC CCGCTGATCC TTTGCGAATA 3500
CGCCCACGCG ATGGGTAACA GTCTTGGCGG TTTCGCTAAA TACTGGCAGG 3550
CGTTTCGTCA GTATCCCCGT TTACAGGGCG GCTTCGTCTG GGACTGGGTG 3600
GATCAGTCGC TGATTAAATA TGATGAAAAC GGCAACCCGT GGTCGGCTTA 3650
CGGCGGTGAT TTTGGCGATA CGCCGAACGA TCGCCAGTTC TGTATGAACG 3700
GTCTGGTCTT TGCCGACCGC ACGCCGCATC CAGCGCTGAC GGAAGCAAAA 3750
CACCAGCAGC AGTTTTTCCA GTTCCGTTTA TCCGGGCAAA CCATCGAAGT 3800
GACCAGCGAA TACCTGTTCC GTCATAGCGA TAACGAGCTC CTGCACTGGA 3850
TGGTGGCGCT GGATGGTAAG CCGCTGGCAA GCGGTGAAGT GCCTCTGGAT 3900
GTCGCTCCAC AAGGTAAACA GTTGATTGAA CTGCCTGAAC TACCGCAGCC 3950
GGAGAGCGCC GGGCAACTCT GGCTCACAGT ACGCGTAGTG CAACCGAACG 4000
CGACCGCATG GTCAGAAGCC GGGCACATCA GCGCCTGGCA GCAGTGGCGT 4050
CTGGCGGAAA ACCTCAGTGT GACGCTCCCC GCCGCGTCCC ACGCCATCCC 4100
GCATCTGACC ACCAGCGAAA TGGATTTTTG CATCGAGCTG GGTAATAAGC 4150
GTTGGCAATT TAACCGCCAG TCAGGCTTTC TTTCACAGAT GTGGATTGGC 4200
GATAAAAAAC AACTGCTGAC GCCGCTGCGC GATCAGTTCA CCCGTGCACC 4250
GCTGGATAAC GACATTGGCG TAAGTGAAGC GACCCGCATT GACCCTAACG 4300
CCTGGGTCGA ACGCTGGAAG GCGGCGGGCC ATTACCAGGC CGAAGCAGCG 4350
TTGTTGCAGT GCACGGCAGA TACACTTGCT GATGCGGTGC TGATTACGAC 4400
CGCTCACGCG TGGCAGCATC AGGGGAAAAC CTTATTTATC AGCCGGAAAA 4450

CCTACCGGAT TGATGGTAGT GGTCAAATGG CGATTACCGT TGATGTTGAA 4500
GTGGCGAGCG ATACACCGCA TCCGGCGCGG ATTGGCCTGA ACTGCCAGCT 4550
GGCGCAGGTA GCAGAGCGGG TAAACTGGCT CGGATTAGGG CCGCAAGAAA 4600
ACTATCCCGA CCGCCTTACT GCCGCCTGTT TTGACCGCTG GGATCTGCCA 4650
TTGTCAGACA TGTATACCCC GTACGTCTTC CCGAGCGAAA ACGGTCTGCG 4700
CTGCGGGACG CGCGAATTGA ATTATGGCCC ACACCAGTGG CGCGGCGACT 4750
TCCAGTTCAA CATCAGCCGC TACAGTCAAC AGCAACTGAT GGAAACCAGC 4800
CATCGCCATC TGCTGCACGC GGAAGAAGGC ACATGGCTGA ATATCGACGG 4850
TTTCCATATG GGGATTGGTG GCGACGACTC CTGGAGCCCG TCAGTATCGG 4900
CGGAATTACA GCTGAGCGCC GGTCGCTACC ATTACCAGTT GGTCTGGTGT 4950
CAAAAATAAT AATAACCGGG CAGGCCATGT CTGCCCGTAT TTCGCGTAAG 5000
GAAATCCATT ATGTACTATT TAAAAAACAC AAACTTTTGG ATGTTCGGTT 5050
TATTCTTTTT CTTTTACTTT TTTATCATGG GAGCCTACTT CCCGTTTTTC 5100
CCGATTTGGC TACATGACAT CAACCATATC AGCAAAAGTG ATACGGGTAT 5150
TATTTTTGCC GCTATTTCTC TGTTCTCGCT ATTATTCCAA CCGCTGTTTG 5200
GTCTGCTTTC TGACAAACTC GGCCTCGACT CTAGGCGGCC GCGGGGATCC 5250
AGACATGATA AGATACATTG ATGAGTTTGG ACAAACCACA ACTAGAATGC 5300
AGTGAAAAAA ATGCTTTATT TGTGAAATTT GTGATGCTAT TGCTTTATTT 5350
GTAACCATTA TAAGCTGCAA TAAACAAGTT AACAACAACA ATTGCATTCA 5400
TTTTATGTTT CAGGTTCAGG GGGAGGTGTG GGAGGTTTTT TCGGATCCTC 5450
TAGAGTCGAC GACGCGAGGC TGGATGGCCT TCCCCATTAT GATTCTTCTC 5500
GCTTCCGGCG GCATCGGGAT GCCCGCGTTG CAGGCCATGC TGTCCAGGCA 5550
GGTAGATGAC GACCATCAGG GACAGCTTCA AGGATCGCTC GCGGCTCTTA 5600
CCAGCCTAAC TTCGATCACT GGACCGCTGA TCGTCACGGC GATTTATGCC 5650
GCCTCGGCGA GCACATGGAA CGGGTTGGCA TGGATTGTAG GCGCCGCCCT 5700
ATACCTTGTC TGCCTCCCCG CGTTGCGTCG CGGTGCATGG AGCCGGGCCA 5750

CCTCGACCTG AATGGAAGCC GGCGGCACCT CGCTAACGGA TTCACCACTC 5800

CAAGAATTGG AGCCAATCAA TTCTTGCGGA GAACTGTGAA TGCGCAAACC 5850

AACCCTTGGC AGAACATATC CATCGCGTCC GCCATCTCCA GCAGCCGCAC 5900

GCGGCGCATC TCGGGCAGCG TTGGGTCCTG GCCACGGGTG CGCATGATCG 5950

TGCTCCTGTC GTTGAGGACC CGGCTAGGCT GGCGGGGTTG CCTTACTGGT 6000

TAGCAGAATG AATCACCGAT ACGCGAGCGA ACGTGAAGCG ACTGCTGCTG 6050

CAAAACGTCT GCGACCTGAG CAACAACATG AATGGTCTTC GGTTTCCGTG 6100

TTTCGTAAAG TCTGGAAACG CGGAAGTCAG CGCCCTGCAC CATTATGTTC 6150

CGGATCTGCA TCGCAGGATG CTGCTGGCTA CCCTGTGGAA CACCTACATC 6200

TGTATTAACG AAGCCTTTCT CAATGCTCAC GCTGTAGGTA TCTCAGTTCG 6250

GTGTAGGTCG TTCGCTCCAA GCTGGGCTGT GTGCACGAAC CCCCCGTTCA 6300

GCCCGACCGC TGCGCCTTAT CCGGTAACTA TCGTCTTGAG TCCAACCCGG 6350

TAAGACACGA CTTATCGCCA CTGGCAGCAG CCACTGGTAA CAGGATTAGC 6400

AGAGCGAGGT ATGTAGGCGG TGCTACAGAG TTCTTGAAGT GGTGGCCTAA 6450

CTACGGCTAC ACTAGAAGGA CAGTATTTGG TATCTGCGCT CTGCTGAAGC 6500

CAGTTACCTT CGGAAAAAGA GTTGGTAGCT CTTGATCCGG CAAACAAACC 6550

ACCGCTGGTA GCGGTGGTTT TTTTGTTTGC AAGCAGCAGA TTACGCGCAG 6600

AAAAAAAGGA TCTCAAGAAG ATCCTTTGAT CTTTTCTACG GGGTCTGACG 6650

CTCAGTGGAA CGAAAACTCA CGTTAAGGGA TTTTGGTCAT GAGATTATCA 6700

AAAAGGATCT TCACCTAGAT CCTTTTAAAT TAAAAATGAA GTTTTAAATC 6750

AATCTAAAGT ATATATGAGT AAACTTGGTC TGACAGTTAC CAATGCTTAA 6800

TCAGTGAGGC ACCTATCTCA GCGATCTGTC TATTTCGTTC ATCCATAGTT 6850

GCCTGACTCC CCGTCGTGTA GATAACTACG ATACGGGAGG GCTTACCATC 6900

TGGCCCCAGT GCTGCAATGA TACCGCGAGA CCCACGCTCA CCGGCTCCAG 6950

ATTTATCAGC AATAAACCAG CCAGCCGGAA GGGCCGAGCG CAGAAGTGGT 7000

CCTGCAACTT TATCCGCCTC CATCCAGTCT ATTAATTGTT GCCGGGAAGC 7050

TAGAGTAAGT AGTTCGCCAG TTAATAGTTT GCGCAACGTT GTTGCCATTG 7100

CTGCAGGCAT CGTGGTGTCA CGCTCGTCGT TTGGTATGGC TTCATTCAGC 7150

TCCGGTTCCC AACGATCAAG GCGAGTTACA TCATCCCCCA TGTTGTGCAA 7200

AAAAGCGGTT AGCTCCTTCG GTCCTCCGAT CGTTGTCAGA AGTAAGTTGG 7250

CCGCAGTGTT ATCACTCATG GTTATGCCAG CACTGCATAA TTCTCTTACT 7300

GTCATGCCAT CCGTAAGATG CTTTTCTGTG ACTGGTGAGT ACTCAACCAA 7350

GTCATTCTGA GAATAGTGTA TGCGGCGACC GAGTTGCTCT TGCCCGGCGT 7400

CAACACGGGA TAATACCGCG CCACATAGCA CAACTTTAAA AGTGCTCATC 7450

ATTGGAAAAC GTTCTTCGGG GCGAAAACTC TCAAGGATCT TACCGCTGTT 7500

GAGATCCAGT TCGATGTAAC CCACTCGTGC ACCCAACTGA TCTTCAGCAT 7550

CTTTTACTTT CACCAGCGTT TCTGGGTGAG CAAAAACAGG AAGGCAAAAT 7600

GCCGCAAAAA AGGGAATAAG GGCGACACGG AAATGTTGAA TACTCATACT 7650

CTTCCTTTTT CAATATTATT GAAGCATTTA TCAGGGTTAT TGTCTCATGA 7700

GCGGATACAT ATTTGAATGT ATTTAGAAAA ATAAACAAAT AGGGGTTCCG 7750

CGCACATTTC CCCGAAAAGT GCCACCTGAC GTCTAAGAAA CCATTATTAT 7800

CATGACATTA ACCTATAAAA ATAGGCGTAT CACGAGGCCC TTTCGTCTTC 7850

AA 7852

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9972 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TCTTCCGCTT CCTCGCTCAC TGACTCGCTG CGCTCGGTCG TTCGGCTGCG 50

GCGAGCGGTA TCAGCTCACT CAAAGGCGGT AATACGGTTA TCCACAGAAT 100

CAGGGGATAA CGCAGGAAAG AACATGTGAG CAAAAGGCCA GCAAAAGGCC 150

AGGAACCGTA AAAAGGCCGC GTTGCTGGCG TTTTTCCATA GGCTCCGCCC 200

CCCTGACGAG CATCACAAAA ATCGACGCTC AAGTCAGAGG TGGCGAAACC 250

CGACAGGACT ATAAAGATAC CAGGCGTTTC CCCCTGGAAG CTCCCTCGTG 300

CGCTCTCCTG TTCCGACCCT GCCGCTTACC GGATACCTGT CCGCCTTTCT 350

CCCTTCGGGA AGCGTGGCGC TTTCTCATAG CTCACGCTGT AGGTATCTCA 400

GTTCGGTGTA GGTCGTTCGC TCCAAGCTGG GCTGTGTGCA CGAACCCCCC 450

GTTCAGCCCG ACCGCTGCGC CTTATCCGGT AACTATCGTC TTGAGTCCAA 500

CCCGGTAAGA CACGACTTAT CGCCACTGGC AGCAGCCACT GGTAACAGGA 550

TTAGCAGAGC GAGGTATGTA GGCGGTGCTA CAGAGTTCTT GAAGTGGTGG 600

CCTAACTACG GCTACACTAG AAGAACAGTA TTTGGTATCT GCGCTCTGCT 650

GAAGCCAGTT ACCTTCGGAA AAAGAGTTGG TAGCTCTTGA TCCGGCAAAC 700

AAACCACCGC TGGTAGCGGT GGTTTTTTTG TTTGCAAGCA GCAGATTACG 750

CGCAGAAAAA AAGGATCTCA AGAAGATCCT TTGATCTTTT CTACGGGGTC 800

TGACGCTCAG TGGAACGAAA ACTCACGTTA AGGGATTTTG GTCATGAGAT 850

TATCAAAAAG GATCTTCACC TAGATCCTTT TAAATTAAAA ATGAAGTTTT 900

AAATCAATCT AAAGTATATA TGAGTAAACT TGGTCTGACA GTTACCAATG 950

CTTAATCAGT GAGGCACCTA TCTCAGCGAT CTGTCTATTT CGTTCATCCA 1000

TAGTTGCCTG ACTCCCCGTC GTGTAGATAA CTACGATACG GGAGGGCTTA 1050

CCATCTGGCC CCAGTGCTGC AATGATACCG CCAGACCCAC GCTCACCGGC 1100

TCCAGATTTA TCAGCAATAA ACCAGCCAGC CGGAAGGGCC GAGCGCAGAA 1150

GTGGTCCTGC AACTTTATCC GCCTCCATCC AGTCTATTAA TTGTTGCCGG 1200

GAAGCTAGAG TAAGTAGTTC GCCAGTTAAT AGTTTGCGCA ACGTTGTTGC 1250

CATTGCTACA GGCATCGTGG TGTCACGCTC GTCGTTTGGT ATGGCTTCAT 1300

TCAGCTCCGC TTCCCAACGA TCAAGGCGAG TTACATGATC CCCCATGTTG 1350

TGCAAAAAAG CGGTTAGCTC CTTCGGTCCT CCGATCGTTG TCAGAAGTAA 1400

GTTGGCCGCA GTGTTATCAC TCATGGTTAT GGCAGCACTG CATAATTCTC 1450
TTACTGTCAT GCCATCCGTA AGATGCTTTT CTGTGACTGG TGAGTACTCA 1500
ACCAAGTCAT TCTGAGAATA GTGTATGCGG CGACCGAGTT GCTCTTGCCC 1550
GGCGTCAATA CGGGATAATA CCGCGCCACA TAGCAGAACT TTAAAAGTGC 1600
TCATCATTGG AAAACGTTCT TCGGGGCGAA AACTCTCAAG GATCTTACCG 1650
CTGTTGAGAT CCAGTTCGAT GTAACCCACT CGTGCACCCA ACTGATCTTC 1700
AGCATCTTTT ACTTTCACCA GCGTTTCTGG GTGAGCAAAA ACAGGAAGGC 1750
AAAATGCCGC AAAAAAGGGA ATAAGGGCGA CACGGAAATG TTGAATACTC 1800
ATACTCTTCC TTTTTCAATA TTATTGAAGC ATTTATCAGG GTTATTGTCT 1850
CATGAGCGGA TACATATTTG AATGTATTTA GAAAAATAAA CAAATAGGGG 1900
TTCCGCGCAC ATTTCCCCGA AAAGTGCCAC CTGACGTCTA AGAAACCATT 1950
ATTATCATGA CATTAACCTA TAAAAATAGG CGTATCACGA GGCCCTTTCG 2000
TCTCGCGCGT TTCGGTGATG ACGGTGAAAA CCTCTGACAC ATGCAGCTCC 2050
CGGAGACGGT CACAGCTTGT CTGTAAGCGG ATGCCGGGAG CAGACAAGCC 2100
CGTCAGGGCG CGTCAGCGGG TGTTGGCGGG TGTCGGGGCT GGCTTAACTA 2150
TGCGGCATCA GAGCAGATTG TACTGAGAGT GCACCATAAA ATTGTAAACG 2200
TTAATATTTT GTTAAAATTC GCGTTAAATT TTTGTTAAAT CAGCTCATTT 2250
TTTAACCAAT AGGCCGAAAT CGGCAAAATC CCTTATAAAT CAAAAGAATA 2300
GCCCGAGATA GGGTTGAGTG TTGTTCCAGT TTGGAACAAG AGTCCACTAT 2350
TAAAGAACGT GGACTCCAAC GTCAAAGGGC GAAAAACCGT CTATCAGGGC 2400
GATGGCCCAC TACGTGAACC ATCACCCAAA TCAAGTTTTT TGGGGTCGAG 2450
GTGCCGTAAA GCACTAAATC GGAACCCTAA AGGGAGCCCC CGATTTAGAG 2500
CTTGACGGGG AAAGCCGGCG AACGTGGCGA GAAAGGAAGG GAAGAAAGCG 2550
AAAGGAGCGG GCGCTAGGGC GCTGGCAAGT GTAGCGGTCA CGCTGCGCGT 2600
AACCACCACA CCCGCCGCGC TTAATGCGCC GCTACAGGGC GCGTACTATG 2650
GTTGCTTTGA CGTATGCGGT GTGAAATACC GCACAGATGC GTAAGGAGAA 2700

AATACCGCAT CAGGCGCCAT TCGCCATTCA GGCTGCGCAA CTGTTGGGAA 2750
GGGCGATCGG TGCGGGCCTC TTCGCTATTA CGCCAGCTGG CGAAAGGGGG 2800
ATGTGCTGCA AGGCGATTAA GTTGGGTAAC GCCAGGGTTT TCCCAGTCAC 2850
GACGTTGTAA AACGACGGCC AGTGCCAAGC TAAAGGTGCA CGGCCCACGT 2900
GGCCACTAGT ACTTCTCGAG CTCTGTACAT GTCCGCGGTC GCGACGTACG 2950
CGTATCGATG GCGCCAGCTG CAGGCGGCCG CCATATGCAT CCTAGGCCTA 3000
TTAATATTCC GGAGTATACG TAGCCGGCTA ACGTTAACAA CCGGTACCTC 3050
TAGAACTATA GCTAGCCAAT TCCATCATCA ATAATATACC TTATTTTGGA 3100
TTGAAGCCAA TATGATAATG AGGGGGTGGA GTTTGTGACG TGGCGCGGGG 3150
CGTGGGAACG GGGCGGGTGA CGTAGGTTTT AGGGCGGAGT AACTTGTATG 3200
TGTTGGGAAT TGTAGTTTTC TTAAAATGGG AAGTTACGTA ACGTGGGAAA 3250
ACGGAAGTGA CGATTTGAGG AAGTTGTGGG TTTTTTGGCT TTCGTTTCTC 3300
GGCGTAGGTT CGCGTGCGGT TTTCTGGGTG TTTTTTGTGG ACTTTAACCG 3350
TTACGTCATT TTTTAGTCCT ATATATACTC GCTCTGCACT TGGCCCTTTT 3400
TTACACTGTG ACTGATTGAG CTGGTGCCGT GTCGAGTGGT GTTTTTTTAA 3450
TAGGTTTTCT TTTTTACTGG TAAGGCTGAC TGTTAGGCTG CCGCTGTGAA 3500
GCGCTGTATG TTGTTCTGGA GCGGGAGGGT GCTATTTTGC CTAGGCAGGA 3550
GGGTTTTTCA GGTGTTTATG TGTTTTTCTC TCCTATTAAT TTTGTTATAC 3600
CTCCTATGGG GGCTGTAATG TTGTCTCTAC GCCTGCGGGT ATGTATTCCC 3650
CCCAAGCTTG CATGCCTGCA GGTCGACTCT AGAGGATCCG AAAAAACCTC 3700
CCACACCTCC CCCTGAACCT GAAACATAAA ATGAATGCAA TTGTTGTTGT 3750
TAACTTGTTT ATTGCAGCTT ATAATGGTTA CAAATAAAGC AATAGCATCA 3800
CAAATTTCAC AAATAAAGCA TTTTTTTCAC TGCATTCTAG TTGTGGTTTG 3850
TCCAAACTCA TCAATGTATC TTATCATGTC TGGATCCCCC TAGCTTGCCA 3900
AACCTACAGG TGGGGTCTTT CATTCCCCCC TTTTTCTGGA GACTAAATAA 3950
AATCTTTTAT TTTATCTATG GCTCGTACTC TATAGGCTTC AGCTGGTGAT 4000

ATTGTTGAGT CAAAACTAGA GCCTGGACCA CTGATATCCT GTCTTTAACA 4050

AATTGGACTA ATCGCGGGAT CAGCCAATTC CATGAGCAAA TGTCCCATGT 4100

CAACATTTAT GCTGCTCTCT AAAGCCTTGT ATCTTGCATC TCTTCTTCTG 4150

TCTCCTCTTT CAGAGCAGCA ATCTGGGGCT TAGACTTGCA CTTGCTTGAG 4200

TTCCGGTGGG GAAAGAGCTT CACCCTGTCG GAGGGGCTGA TGGCTTGCCG 4250

GAAGAGGCTC CTCTCGTTCA GCAGTTTCTG GATGGAATCG TACTGCCGCA 4300

CTTTGTTCTC TTCTATGACC AAAAATTGTT GGCATTCCAG CATTGCTTCT 4350

ATCCTGTGTT CACAGAGAAT TACTGTGCAA TCAGCAAATG CTTGTTTTAG 4400

AGTTCTTCTA ATTATTTGGT ATGTTACTGG ATCCAAATGA GCACTGGGTT 4450

CATCAAGCAG CAAGATCTTC GCCTTACTGA GAACAGATCT AGCCAAGCAC 4500

ATCAACTGCT TGTGGCCATG GCTTAGGACA CAGCCCCCAT CCACAAGGAC 4550

AAAGTCAAGC TTCCCAGGAA ACTGTTCTAT CACAGATCTG AGCCCAACCT 4600

CATCTGCAAC TTTCCATATT TCTTGATCAC TCCACTGTTC ATAGGGATCC 4650

AAGTTTTTTC TAAATGTTCC AGAAAAAATA AATACTTTCT GTGGTATCAC 4700

TCCAAAGGCT TTCCTCCACT GTTGCAAAGT TATTGAATCC CAAGACACAC 4750

CATCGATCTG GATTTCTCCT TCAGTGTTCA GTAGTCTCAA AAAAGCTGAT 4800

AACAAAGTAC TCTTCCCTGA TCCAGTTCTT CCCAAGAGGC CCACCCTCTG 4850

GCCAGGACTT ATTGAGAAGG AAATGTTCTC TAATATGGCA TTTCCACCTT 4900

CTGTGTATTT TGCTGTGAGA TCTTTGACAG TCATTTGGCC CCCTGAGGGC 4950

CAGATGTCAT CTTTCTTCAC GTGTGAATTC TCAATAATCA TAACTTTCGA 5000

GAGTTGGCCA TTCTTGTATG GTTTGGTTGA CTTGGTAGGT TTACCTTCTG 5050

TTGGCATGTC AATGAACTTA AAGACTCGGC TCACAGATCG CATCAAGCTA 5100

TCCACATCTA TGCTGGAGTT TACAGCCCAC TGCAATGTAC TCATGATATT 5150

CATGGCTAAA GTCAGGATAA TACCAACTCT TCCTTCTCCT TCTCCTGTTG 5200

TTAAAATGGA AATGAAGGTA ACAGCAATGA AGAAGATGAC AAAAATCATT 5250

TCTATTCTCA TTTGGAACCA GCGCAGTGTT GACAGGTACA AGAACCAGTT 5300

GGCAGTATGT AAATTCAGAG CTTTGTGGAA CAGAGTTTCA AAGTAAGGCT 5350
GCCGTCCGAA GGCACGAAGT GTCCATAGTC CTTTTAAGCT TGTAACAAGA 5400
TGAGTGAAAA TTGGACTCCT GCCTTCAGAT TCCAGTTGTT TGAGTTGCTG 5450
TGAGGTTTGG AGGAAATATG CTCTCAACAT AATAAAAGCC ACTATCACTG 5500
GCACTGTTGC AACAAAGATG TAGGGTTGTA AAACTGCGAC AACTGCTATA 5550
GCTCCAATCA CAATTAATAA CAACTGGATG AAGTCAAATA TGGTAAGAGG 5600
CAGAAGGTCA TCCAAAATTG CTATATCTTT GGAGAATCTA TTAAGAATCC 5650
CACCTGCTTT CAACGTGTTG AGGGTTGACA TAGGTGCTTG AAGAACAGAA 5700
TGTAACATTT TGTGGTGTAA AATTTTCGAC ACTGTGATTA GAGTATGCAC 5750
CAGTGGTAGA CCTCTGAAGA ATCCCATAGC AAGCAAAGTG TCGGCTACTC 5800
CCACGTAAAT GTAAAACACA TAATACGAAC TGGTGCTGGT GATAATCACT 5850
GCATAGCTGT TATTTCTACT ATGAGTACTA TTCCCTTTGT CTTGAAGAGG 5900
AGTGTTTCCA AGGAGCCACA GCACAACCAA AGAAGCAGCC ACCTCTGCCA 5950
GAAAAATTAC TAAGCACCAA ATTAGCACAA AAATTAAGCT CTTGTGGACA 6000
GTAATATATC GAAGGTATGT GTTCCATGTA GTCACTGCTG GTATGCTCTC 6050
CATATCATCA AAAAAGCACT CCTTTAAGTC TTCTTCGTTA ATTTCTTCAC 6100
TTATTTCCAA GCCAGTTTCT TGAGATAACC TTCTTGAATA TATATCCAGT 6150
TCAGTCAAGT TTGCCTGAGG GGCCAGTGAC ACTTTTCGTG TGGATGCTGT 6200
TGTCTTTCGG TGAATGTTCT GACCTTGGTT AACTGAGTGT GTCATCAGGT 6250
TCAGGACAGA CTGCCTCCTT CGTGCCTGAA GCGTGGGGCC AGTGCTGATC 6300
ACGCTGATGC GAGGCAGTAT CGCCTCTCCC TGCTCAGAAT CTGGTACTAA 6350
GGACAGCCTT CTCTCTAAAG GCTCATCAGA ATCCTCTTCG ATGCCATTCA 6400
TTTGTAAGGG AGTCTTTTGC ACAATGGAAA ATTTTCGTAT AGAGTTGATT 6450
GGATTGAGAA TAGAATTCTT CCTTTTTTCC CCAAACTCTC CAGTCTGTTT 6500
AAAAGATTGT TTTTTTGTTT CTGTCCAGGA GACAGGAGCA TCTCCTTCTA 6550
ATGAGAAACG GTGTAAGGTC TCAGTTAGGA TTGAATTTCT TCTTTCTGCA 6600

CTAAATTGGT CGAAAGAATC ACATCCCATG AGTTTTGAGC TAAAGTCTGG 6650
CTGTAGATTT TGGAGTTCTG AAAATGTCCC ATAAAAATAG CTGCTACCTT 6700
CATGCAAAAT TAATATTTTG TCAGCTTTCT TTAAATGTTC CATTTTAGAA 6750
GTGACCAAAA TCCTAGTTTT GTTAGCCATC AGTTTACAGA CACAGCTTTC 6800
AAATATTTCT TTTTCTGTTA AAACATCTAG GTATCCAAAA GGAGAGTCTA 6850
ATAAATACAA ATCAGCATCT TTGTATACTG CTCTTGCTAA AGAAATTCTT 6900
GCTCGTTGAC CTCCACTCAG TGTGATTCCA CCTTCTCCAA GAACTATATT 6950
GTCTTTCTCT GCAAACTTGG AGATGTCCTC TTCTAGTTGG CATGCTTTGA 7000
TGACGCTTCT GTATCTATAT TCATCATAGG AAACACCAAA GATGATATTT 7050
TCTTTAATGG TGCCAGGCAT AATCCAGGAA AACTGAGAAC AGAATGAAAT 7100
TCTTCCACTG TGCTTAATTT TACCCTCTGA AGGCTCCAGT TCTCCCATAA 7150
TCATCATTAG AAGTGAAGTC TTGCCTGCTC CAGTGGATCC AGCAACCGCC 7200
AACAACTGTC CTCTTTCTAT CTTGAAATTA ATATCTTTCA GGACAGGAGT 7250
ACCAAGAAGT GAGAAATTAC TGAAGAAGAG GCTGTCATCA CCATTAGAAG 7300
TTTTTCTATT GTTATTGTTT TGTTTTGCTT TCTCAAATAA TTCCCCAAAT 7350
CCCTCCTCCC AGAAGGCTGT TACATTCTCC ATCACTACTT CTGTAGTCGT 7400
TAAGTTATAT TCCAATGTCT TATATTCTTG CTTTTGTAAG AAATCCTGTA 7450
TTTTGTTTAT TGCTCCAAGA GAGTCATACC ATGTTTGTAC AGCCCAGGGA 7500
AATTGCCGAG TGACCGCCAT GCGCAGAACA ATGCAGAATG AGATGGTGGT 7550
GAATATTTTC CGGAGGATGA TTCCTTTGAT TAGTGCATAG GGAAGCACAG 7600
ATAAAAACAC CACAAAGAAC CCTGAGAAGA AGAAGGCTGA GCTATTGAAG 7650
TATCTCACAT AGGCTGCCTT CCGAGTCAGT TTCAGTTCTG TTTGTCTTAA 7700
GTTTTCAATC ATTTTTTCCA TTGCTTCTTC CCAGCAGTAT GCCTTAACAG 7750
ATTGGATGTT CTCGATCATT TCTGAGGTAA TCACAAGTCT TTCACTGATC 7800
TTCCCAGCTC TCTGATCTCT GTACTTCATC ATCATTCTCC CTAGCCCAGC 7850
CTGAAAAAGG GCAAGGACTA TCAGGAAACC AAGTCCACAG AAGGCAGACG 7900

CCTGTAACAA CTCCCAGATT AGCCCCATGA GGAGTGCCAC TTGCAAAGGA 7950
GCGATCCACA CGAAATGTGC CAATGCAAGT CCTTCATCAA ATTTGTTCAG 8000
GTTGTTGGAA AGGAGACTAA CAAGTTGTCC AATACTTATT TTATCTAGAA 8050
CACGGCTTGA CAGCTTTAAA GTCTTCTTAT AAATCAAACT AAACATAGCT 8100
ATTCTCATCT GCATTCCAAT GTGATGAAGG CCAAAAATGG CTGGGTGTAG 8150
GAGCAGTGTC CTCACAATAA AGAGAAGGCA TAAGCCTATG CCTAGATAAA 8200
TCGCGATAGA GCGTTCCTCC TTGTTATCCG GGTCATAGGA AGCTATGATT 8250
CTTCCCAGTA AGAGAGGCTG TACTGCTTTG GTGACTTCCC CTAAATATAA 8300
AAAGATTCCA TAGAACATAA ATCTCCAGAA AAAACATCGC CGAAGGGCAT 8350
TAATGAGTTT AGGATTTTTC TTTGAAGCCA GCTCTCTATC CCATTCTCTT 8400
TCCAATTTTT CAGATAGATT GTCAGCAGAA TCAACAGAAG GGATTTGGTA 8450
TATGTCTGAC AATTCCAGGC GCTGTCTGTA TCCTTTCCTC AAAATTGGTC 8500
TGGTCCAGCT GAAAAAAAGT TTGGAGACAA CGCTGGCCTT TTCCAGAGGC 8550
GACCTCTGCA TGGTCTCTCG GGCGCTGGGG TCCCTGCTAG GGCCGTCTGG 8600
GCTCAAGCTC CTAATGCCAA AGGAATTCCT GCAGCCCGGG GGATCCACTA 8650
GTTCTAGAGC GGCCGCCACC GCGGTGGCTG ATCCCGCTCC CGCCCGCCGC 8700
GCGCTTCGCT TTTTATAGGG CCGCCGCCGC CGCCGCCTCG CCATAAAAGG 8750
AAACTTTCGG AGCGCGCCGC TCTGATTGGC TGCCGCCGCA CCTCTCCGCC 8800
TCGCCCCGCC CCGCCCCTCG CCCCGCCCCG CCCCGCCTGG CGCGCGCCCC 8850
CCCCCCCCCC CCGCCCCCAT CGCTGCACAA AATAATTAAA AAATAAATAA 8900
ATACAAAATT GGGGGTGGGG AGGGGGGGGA GATGGGGAGA GTGAAGCAGA 8950
ACGTGGCCTC GAGTAGATGT ACTGCCAAGT AGGAAAGTCC CATAAGGTCA 9000
TGTACTGGGC ATAATGCCAG GCGGGCCATT TACCGTCATT GACGTCAATA 9050
GGGGGCGTAC TTGGCATATG ATACACTTGA TGTACTGCCA AGTGGGCAGT 9100
TTACCGTAAA TACTCCACCC ATTGACGTCA ATGGAAAGTC CCTATTGGCG 9150
TTACTATGGG AACATACGTC ATTATTGACG TCAATGGGCG GGGGTCGTTG 9200

GGCGGTCAGC CAGGCGGGCC ATTTACCGTA AGTTATGTAA CGACCTGCAG 9250

GCTGATCTCC CTAGACAAAT ATTACGCGCT ATGAGTAACA CAAAATTATT 9300

CAGATTTCAC TTCCTCTTAT TCAGTTTTCC CGCGAAAATG GCCAAATCTT 9350

ACTCGGTTAC GCCCAAATTT ACTACAACAT CCCCCTAAAA CCGCGCGAAA 9400

ATTGTCACTT CCTGTGTACA CCGGCGCACA CCAAAAACGT CACTTTTGCC 9450

ACATCCGTCG CTTACATGTG TTCCGCCACA CTTGCAACAT CACACTTCCG 9500

CCACACTACT ACGTCACCCG CCCCGTTCCC ACGCCCCGCG CCACGTCACA 9550

AACTCCACCC CCTCATTATC ATATTGGCTT CAATCCAAAA TAAGGTATAT 9600

TATTGATGAT GCTAGCATGC GCAAATTTAA AGCGCTGATA TCGATCGCGC 9650

GCAGATCTGT CATGATGATC ATTGCAATTG GATCCATATA TAGGGCCCGG 9700

GTTATAATTA CCTCAGGTCG ACGTCCCATG GCCATTCGAA TTCGTAATCA 9750

TGGTCATAGC TGTTTCCTGT GTGAAATTGT TATCCGCTCA CAATTCCACA 9800

CAACATACGA GCCGGAAGCA TAAAGTGTAA AGCCTGGGGT GCCTAATGAG 9850

TGAGCTAACT CACATTAATT GCGTTGCGCT CACTGCCCGC TTTCCAGTCG 9900

GGAAACCTGT CGTGCCAGCT GCATTAATGA ATCGGCCAAC GCGCGGGGAG 9950

AGGCGGTTTG CGTATTGGGC GC 9972

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TAGTAAATTT GGGC 14

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AGTAAGATTT GGCC 14

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AGTGAAATCT GAAT 14

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GAATAATTTT GTGT 14

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CGTAATATTT GTCT 14

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

WANWTTTG 8

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19307 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CCAATTCCAT CATCAATAAT ATACCTTATT TTGGATTGAA GCCAATATGA 50

TAATGAGGGG GTGGAGTTTG TGACGTGGCG CGGGGCGTGG GAACGGGGCG 100

GGTGACGTAG GTTTTAGGGC GGAGTAACTT GTATGTGTTG GGAATTGTAG 150

TTTTCTTAAA ATGGGAAGTT ACGTAACGTG GGAAAACGGA AGTGACGATT 200

TGAGGAAGTT GTGGGTTTTT TGGCTTTCGT TTCTGGGCGT AGGTTCGCGT 250

GCGGTTTTCT GGGTGTTTTT TGTGGACTTT AACCGTTACG TCATTTTTTA 300

GTCCTATATA TACTCGCTCT GCACTTGGCC CTTTTTTACA CTGTGACTGA 350

TTGAGCTGGT GCCGTGTCGA GTGGTGTTTT TTTAATAGGT TTTCTTTTTT 400

ACTGGTAAGG CTGACTGTTA GGCTGCCGCT GTGAAGCGCT GTATGTTGTT 450

CTGGAGCGGG AGGGTGCTAT TTTGCCTAGG CAGGAGGGTT TTTCAGGTGT 500

TTATGTGTTT TTCTCTCCTA TTAATTTTGT TATACCTCCT ATGGGGGCTG 550

TAATGTTGTC TCTACGCCTG CGGGTATGTA TACCCCCCAA GCTTGCATGC 600

CTGCAGGTCG ACTCTAGAGG ATCCGAAAAA ACCTCCCACA CCTCCCCCTG 650

AACCTGAAAC ATAAAATGAA TGCAATTGTT GTTGTTAACT TGTTTATTGC 700

AGCTTATAAT GGTTACAAAT AAAGCAATAG CATCACAAAT TTCACAAATA 750

AAGCATTTTT TTCACTGCAT TCTAGTTGTG GTTTGTCCAA ACTCATCAAT 800

GTATCTTATC ATGTCTGGAT CCCCGCGGCC GCTCTAGAAC TAGTGGATCC 850

CCCGGGCTGC AGGAATTCCG TAACATAACT GCGTGCTTTA TTGAGATACA 900

CAGTAAAGCA GTAATATAAT ACAATAGTAA GGCATATATT TGGTGAAATC 950

TGATATGTTG TGAAAATGCA GTAAAACTGA AGTTTAAAAA AATAATTAGT 1000

AAATGTTACA GTGTTGGTGT TAAAACACAA TCTATTATGA TACTCAAGTA 1050

AGAGTCCAGT ACCTGGAGAC AATGATGATA CATGCCATGT GATGATTATG 1100

CTTCAGTTAC ACTGATTATG ATTTACACTT TAATACTTGA TGGTTATAAA 1150

GAACATGAAA TGATGTCCAA ATTATGCTTA AAATCAGCAA TAAAGCTCTC 1200

AGTTTTTATT CAAATATTTT GATAGATTCA CTCCAGAACT AATATCTAAA 1250

AGATAAAACG AAAAGATTAA AACAAAACTA TGCACTCTAT CTACCTTGGA 1300

TTTTAGAATG AAACTTAAAA CTTCTTAGTA GGAAAGGAAC CCCTTGTTTT 1350

AAATCTTGGT GAAAACAAAT CCTTGGATAA AGAAAATGCC CAGTGCCACA 1400

TAAAGGAGAG AGAGAGAGAA AAGCAAGACC AGAACCAAAT TTCAATTTGT 1450

TATCTTAGAG CTTTGGGTTT TCTTTTGGAA ATTATAAATG AAAAAAGGAA 1500

ACTGGTGTCC ACACAACAGA CAAGTGGTGA AGTTGTGAAA TTAGGTGTGC 1550

ACAATTACTA GAAACACCCC AAAACCAAAG TGAGGTAGAA ATAGCATGAG 1600

AAGCTGTGTT TGATGTTAAT TACAATTAAT AATGGACAAA ACCCACTCGC 1650

TAGAAGTTAA TTACACTTGA CGTTAGAGGT AACAGATTTG CAAAATGATA 1700

GGACAGTGAT TTCTATTGAG AGAATGCTCT TTAAATGCTA AGAAGAAGAA 1750

ACTGGCATGA GAGGAGTAAA GCTCTTCCTA GCAGTCCTTA GCTTTCTGTT 1800

GCACTTTTTC TCCTGGTTCA ATGACTTGCA TTTGTTTAGA CATTTCAGCC 1850

CGTCAACTAG ACCAGAGAGT TTGGAGACGC TTTTGCTCTC AAAACTTTCC 1900

AACCACTGTG CCTTCTCACC CACAATCCTG TGTGGAGTTA CTTGCAGGGA 1950

AACCAATGCA AAGGAGACAA ATGCAGTTCA TGGGCTTCTG GACTGATATT 2000

CACCAGGGTC ACAATGTGAT TGGGTTACTT TCTTAACAGT AATCCTAAGT 2050

CTTGCAGCAT TAAAAAAAAA AATCATCACA ATGAAGAAAA AAAAACCCAA 2100

AAAATCTAAA ATCTAAAATT CATCATCATC ATCAACAACA ACAACAACAA 2150

CAACAACAAA ACCACCCACT TCAGGTTGAG TTTATGAAGA GGGCAGAACA 2200

ATTTAGTTGT AATTATAGAG ATGTTTATAT GTATAGTTGT AAATATTCAT 2250

CCATTCTTTT ACAGAGTTGT TGCTCCCCTC ATATAAATTG ACTGAGGAGC 2300

CGCAACCTTT AGCTCCTACC ATCTTCCTCC TACTGTCTGG GAGTTAAAAA 2350

TGTCATCTGA TGTTCTATTG CAGAAACATC ATTAAATATA ACCCAACAGT 2400

AGGAAGTTGA ATATATCAGC CAACAAATTA CTATGATAGT AAGTCCTGTG 2450

TATTCATTCG CATGTTCCTT GAAAAAAATG AATCCTCTAG CTCTCAGTGG 2500

AAAGTTTAAA ACTAGAAACA TCTGGAGCCC TAGACAATAT TTTAGTGTGG 2550

CGGTAGTCTC CTGGCTTTGG GCTCCAGGGA AAATTCACTC TTGCCCAAGC 2600

AGATAAGCCC AGATGACTAG AAGCAATTTC CATTAGGAAG TGGCAAGAAC 2650

ATTTGAAGAA GTAACTTCAT ATCTATTTAT CTATATACCT ATAGTATTTA 2700

TATACTTGTA GACATATAGA TGTATAAAAT GAAAGCCCAT AGCCAGCCCC 2750

ACTCAGTCAA CAATTCTCAA AAGAGCAATA TGAAGCAGTC ATTTGGTGGG 2800

GTTCGTATGC AAGAAAATAA AAAAACGTCA TGAATTCCAT ATGAATACCA 2850

CGCTAAAGTA ATGCAAAACA ATGTGCTGCC TCAGTGTGTG TGTGTGTGTG 2900

TGTGTGTGTG GTGGGTTCGT GCATGTATGT GTGCGTGTGT GTGTGTGTGT 2950

GTGTGTGTGT GTGTGTGTGC GTGTGTGTTT GTTTAGGGGT TTTTATAAAC 3000

AACTTTTTTT ATAAAGCACA CTTTAGTTTA CAATCTCTCT TTATAACTGT 3050
TATAAATTTT TAAACAACCC AAAATGCGTT CCATATAAAG AAATGGCAAG 3100
TTATTTAGCT ATCAAGATTT TACATGTTTT CTTTTAACTT TTTTGTACAA 3150
TTGCATAGAC GTGTAAAACC TGCCATTGTT AACAAAACAA TAACAGACTT 3200
AGAAACTACT GAAATCTACA GTATAGTACC ACTACCCTTC ACAAAAATAT 3250
AGATTTTATT TCTTGTAAAC TCTTACTGTC TAATCCTCTT TGTTGTACGA 3300
ATATTATAAA AACCATGCGG GAATCAGGAG TTGTAAAACA TTTATTCTGC 3350
TCCTTCTTCA TCTGTCATGA CTGAAACTAA GGACTCCATC GCTCTGCCCA 3400
AATCATCTGC CATGTGGAAA AGGCTTCCTA CATTGTGTCC TCTCTCATTG 3450
GCTTTCCGGG GGCATTTCTT CCTCTTGAAC TAGGGAAGGA GTTGTTGAGT 3500
TGCTCCATCA CTTCTTCTAA CCCTGTGCTT GTGTCCTGGG GAGGACTCAG 3550
AAGATCTTCC TCACCCATAG ATTCTGAAGT TTGACTGCCA ACCACTCGGA 3600
GCAGCATAGG CTGACTGCTA TCTGACCTCT GCAGAGAGGT GGAAGGAGAG 3650
GACACCGTGG TGCCATTCAC CTTAGCTTCA GCCTGGGGCT GCTCCAGGAG 3700
CTGTCTCAGT CTATGTAACT GAGACTCCAG CTGTTTATTG TGGTCTTCCA 3750
GGATTTGCAT CCTGGCTTCC AGGCGTCCTT TGTGTTGGCG CAGTAGCTTA 3800
GCCTCAGCAA TGAGCTCAGC ATCCCTGGGA CTCTGAGGAG AGGTGGGCAT 3850
CATCTCAGGA GGAGATGGCA GTGGAGACAG GCCTTTATGC TCATGCTGCT 3900
GCTTCAGGCG ATCATATTCT GCTTGCAGAT TCCTGTTTTC TTCCTCAAGA 3950
TCTGCTAGGA TTCTCTCTAG CTCCCCTCTT TCCTCACTCT CTAAGGAAAT 4000
CAAGATCTGG GCAGGACTAC GAGGCTGGCT CAGGGGGGAG TCCTGGTTCA 4050
AACTTTGGCA GTAATGCTGG ATTAACAAAT GTTCATCATC TATGCTCTCA 4100
TTAGGAGAGA TGCTATCATT TAGATAAGAT CCATTGCTGT TTTCCATTTC 4150
TGCTAGCCTG CTAGCATAAT GTTCAATGCG TGAATGAGTA TCATCGTGTG 4200
AAAGCTGGGG GGACGAGGCA GGCGCAGAAT CTACTGGCCA GAAGTTGATC 4250
AGAGTAACGG GAGTTTCCAT GTTGTCCCCC TCTAACACAG TCTGCACTGG 4300

CAGGTAGCCC ATTCGGGGAT GCTTCGCAAA ATACCTTTTG GTTCGAAATT 4350

TGTTTTTTAG TACCTTGGCG AAGTCGCGAA CATCTTCTCC GGATGTAGTC 4400

GGAGTGCAAT ACTCTACCAT GGGGTAGTGC ATTTTATGGC CCTTTGCAAC 4450

TCGGCCAGAA AAAAAGCAAC TTTGGCAGAT GTCATAATTA AAATGCTTTA 4500

GGCTTCTGTA CCTGAATCCA ATGATTGGAC ACTCCTTACA GATGTTACAC 4550

TTGGCTTGAT GCTTGGCAGT TTCAGCAGCA GCCACTCTGT GCAAGACGGG 4600

CAGCCACACC ATAGACTGGG GTTCCAGGCG CATCCAGTCA AGGAAGAGAG 4650

CAGCTTCAAT CTCAGGTTTA TTATTGGCAA ATTGGAAGCA GCTCCTGACA 4700

CTCGGCTCAA TGTTACTGCC CCCAAAGGAA GCAACTTCAC CCAACTGTCT 4750

TGGGATTTGA ATAGAATCAT GCAGAAGAAG ACCCAGCCTA CGCTGGTCAC 4800

AAAAGCCAGT TGAACTTGCC ACTTGCTTGA AAAGGTATCT GTACTTGTCT 4850

TCCAAGTGTG CTTTACACAG AGAAATGATG CCAGTTTTAA AAGACAGGAC 4900

ACGGATCCTC CCTGTTCGTC CCGTATCATA AACATTGAGA AGCCAGTTGA 4950

GACACATATC CACACAGAGA GGGACATTGA CCAGATTGTT GTGCTCTTGC 5000

TCCAGACGAT CATAAATTGT AGTCAAACAG TTAATTATCT GCAGGATATC 5050

CATGGGCTGG TCATTTTGCT TGAGGTTGTG CTGGTCCAGG GCATCACATG 5100

CAGCTGACAG GCTCAAGAGA TCCAAGCAAA GGGCCTTCTG GAGCCTTCTG 5150

AGCTTCATGG CAGTCCTATA CGCGGAGAAC CTGACATTAT TCAGGTCAGC 5200

TAAAGACTGG TAGAGCTCTG TCATTTTGGG GTGGTCCCAA CAAGTGGTTT 5250

GGGTCTCGTG GTTGATATAG TAGGGCACTT TGTTTGGTGA GATGGCTCTC 5300

TCCCAGGGAC CCTGAACTGA AGTGGAAAGG AAGTGCTGGG ATGCAGGACC 5350

AAAGTCCCTG TGGGCTTCAT GCAGCTGTCT GACACGGTCC TCCACAGCCA 5400

CCTGTAGAAG CCTCCATCTG GTATTCAGAT CTTCCAAAGT GCTGAGGTTA 5450

TAAGGTGAGA GCTGAATGCC CAGTGTGGTC AGCTGATGTG CAAGGTCATT 5500

GACACGATTG ACATTCTCTT TAAGAGGTGC AATTTCTCCC CGAAGTGCCT 5550

TGACTTTTTC AAGGTGATCT TGCAGAGAGT CAATGAGGAG ATCCCCCACT 5600

GGCTGCCAGG ATCCCTTGAT CACCTCAGCT TGGCGCAACT TGAGGTCCAG 5650
TTCATCGGCA GCTTCCTGAA GTTCCTGGAG TCTTTCAAGA GCTTCATCTA 5700
TTTTTCTCTG CCAATCAGCT GAGCGCAGGT TCAATTTGTC CCATTCAGCG 5750
TTGACCTCTT CAGCCTGCTT TCGTAGGAGC CGAGTGACAT TCTGAGCTCT 5800
TTCTTCAGGA GGCAGTTCTC TGGGCTCCTG GTAGAGTTTC TCTAGTCCTT 5850
CCAAAGGCTG CTCTGTCAGA AATATTCTCA CAGTCTCCAG AGTACTCATG 5900
ATTACAGGTT CTTTAGTTTT CAATTCCCTC TTGAAGGCCC TATGTATATC 5950
ATTCTGCTTC TGAACTGCTG GGAAATCACC ACCGATGGGT GCCTGACGGC 6000
TCAGTTCATC ATCTTTCAGC TGTAGCCAAA CAAGAAGTTC CTGAAGAGAA 6050
AGATGCAAAC GCTTCCACTG GTCAGAACTT GCTTCCAAAT GGGACCTAAT 6100
GTTGAGAGAC TTTTTCTGAA GTTCACTCCA CTTGAAATTC ATGTTATCCA 6150
AACGTCTTTG TAACAGGGGT GCTTCATCCG AACCTTCCAG GGATCTCAGG 6200
ATTTTTTGGC CATTTTCATC AAGATTGTGA TAGATATCTG TGTGAGTTTC 6250
AATTTCTCCT TGGAGATCTT GCCATGGTTT CATCAGCTCT CTGACTCCCC 6300
TGGAGTCTTC TAGGAGCTTC TCCTTACGGG AAGCGTCCTG TAGGACATTG 6350
GCAGTTGTTT CTGCTTCCGT AATCCAGGAA AGAAACTTCT CCAGGTCCAG 6400
AGGGAACTGC TGCAGTAATC TATGAGTTTC TTCCAAAGCA GCCTCTTGCT 6450
CACTTACTCT TTTATGAATG TTTCCCCAAG AAGTATTGAT ATTCTCTGTT 6500
ATCATGTGTA CTTTTCTGGT ATCATCAGCA GAATAGTCCC GAAGAAGTTT 6550
CAGTGCCAAA TCATTTGCCA CGTCTACACT TATCTGCCGT TGACGGAGGT 6600
CTTTGGCCAA CTGCTTGGTT TCTGTGATCT TCTTTTGGAT TGCATCTACT 6650
GTGTGAGGAC CTTCTTTCCA TGAGTCAAGC TTGCCTCTGA CCTGTCCTAT 6700
GACCTGTTCG GCTTCTTCCT TAGCTTCCAG CCATTGTGTT GAATCCTTTA 6750
ACATTTCATT CAACTGTTGT CTCCTGTTCT GCAGCTGTTC TTGAACCTCA 6800
TCCCACTGAA TCTGAATTCT TTCAATTCGA TCAGTAATGA TTGTTCTAGC 6850
TTCTTGATTG CTGGTTTTGT TTTTCAAATT CTGGGCAGCA GTAATGAGTT 6900

CTTCCAATTG GGGGCGTCTC TGTTCCAAAT CTTGCAGTGT TGCCTTCTGT 6950

TTGATGATCA TTTCATTGAT GTCTTCCAGA TCACCCACCA TCACTCTCTG 7000

TGATTTTATA ACTCGATCAA GCAGAGACAG CCAGTCTGTA AGTTCTGTCC 7050

AAGCTCGGTT GAAGTCTGCC AGTGCAGGTA CC[illegible]CCAACAG CAAAGAAGAT 7100

GGCATTTCTA GTTTGGAGAT GACAGTTTCC TTAGTAACCA CAGATTGTGT 7150

CACTAGAGTA ACAGTCTGAC TGGCAGAGGC TCCAGTAGTG CTCAGTCCAG 7200

GGGCACGGTC AGGCTGCTTT GTCCTCAGCT CCCGAAGTAA ATGGTTTACA 7250

GCCTCCCACT CAGACCTCAG ATCTTCTAAC TTCCTCTTCA CTGGCTGAGT 7300

GCTTGGTTTT TCCTTATACA AATGCTGCCC TTTCGACAAA AGCCTTTCCA 7350

CATCCGCTTG TTTACCGTGA ACTGTTACTT CAATCTCCTT TATGTCAAAC 7400

GGTCCTGCCT GACTTGGTTG GTTATAAATT TCCAACTGGT TTCTAATAGG 7450

AGAGACCCAC AGAAGCAGGT GATCCAGCTG CTCTTCAAGC TGCCTAAAAT 7500

CTTTTAAGTG AACCTCAAGC TCTCCTTGTT TCTCAGGTAA AGCTCTGGAG 7550

ACCTTTATCC ACTGGAGATT TGTCTGTTTG AGCTTCTTTT CAAGTTTATC 7600

TTGCTCTTCT GGCCTTATGG GAGCACTTAC AAGTACTGCT CCTCCTGTTT 7650

CATTTAATTG TTTTAGAATT CCCTGGCGCA GGGGCAACTC TTCTGCCAGT 7700

AACTTGACTT GTTCAAGTTG TTCTTTTAGC TGCTGCTCAT CTCCAAGTGG 7750

AGTAATAGCA ATGTTATCTG CTTCTTCCAG CCACAAAACA AATTCATTTA 7800

AATCTCTTTG AAATTCTGAC AAGACATTCT TTTGTTCTTC AATCCTCTTT 7850

CTCCTTTCTG CCAGCTCTTT GCAGATGTCG TGCCACCGCA GACTCAAGCT 7900

TCCTAATTTT TCTTGTAGAA TATTGACATC TGTTTTTGAA GACTGTTGAA 7950

TTATTTCTTC CCCAGTTGCA TTCAGTGTTC TGACAACAGC TTGACGCTGC 8000

CCAATGCCAT CCTGGAGTTC CTTAAGATAC CATTTGTATT TAGCATGTTC 8050

CCAGTTTTCA GGATTTTGTG TCTTTTTGAA AAACTGTTCA ACTTCATTCA 8100

GCCATTGATT AAATACCTTC ATATCATAAT GAAAGTGTCG CCATTTTTCA 8150

ACTGATCTGT CGAATCGCCC TTGTCGTTCC TTGTACATTC TATGAAGTTT 8200

TTCCCCCTGG AAATCCATCT GTGCCACGGC TTCCTGTACT TTCACCTTTT 8250

CCATGGAGGT GGCACTTTGC AAGGCTGCTG TCTTCTTCTT GTGAATAATA 8300

TCAATCCGAC CTGAGATTTG TTGCAAATTG TCTTTTATAT TCTTAAGAGA 8350

CTCCTCTTGC TTAAAAAGAT CTTCAAAATC T.TAGCACAG AGTTCAGGAG 8400

TATTTAGAAG ATGATCAACT TCTGAAAGAG CTTGTAAGAT ATGACTGATC 8450

TCGGTCAAAT AAGTAGAAGG CACATAAGAA ACATCCAAAG GCATATCTTC 8500

AGTCGTCACT ACCATAGTTT CTTCATGGAG AGTGTGAATT TGTGCAAAGT 8550

TGAGTCTTCG AAACTGAGCA AAATTGCTCT CAATTTGCCG CCAGCGCTTG 8600

CTGAGCTGGA TCTGAGTTGG CTCCACTGCC ATTGCGGCCC CATTCTCAGA 8650

CAAGCCCTCA GCTTGCCTGC GCACTGCATT CAGCTCCTCT TTCTTCTTCT 8700

GCAATTCACG ATCAATTTCC TTTAATTTTC TTTCATCTCT GGGTTCAGGT 8750

AGGCTGGCTA ATTTTTTTTC AATTTCATCC AAGCATTTCA GGAGATCATC 8800

AGCCTGCCTC TTGTACTGAT ACCACTGGTG AGAAATTTCT AGGGCCTTTT 8850

TTCTTCTTTG AGACCTCAAA TCCTTGAGAG CATTATGTTT TGTCTGTAAC 8900

AGCTGCTGTT TTATCTTTAT TTCCTCTCGC TTTCTCTCAT CTGTGATTCT 8950

TTGTTGTAAG TTGTCTCCTC TTTGCAACAA TTCATTTACA GTACCCTCAT 9000

TGTCTTCACT CATATCTTTA TTGAAGTCTT CCTCTTTCAG ATTCACCCCC 9050

TGCTGAATTT CAGCCTCCAG TGGTTCAAGC AATTTTTGTA TATCTGAGTT 9100

AAACTGCTCC AATTCCTTCA AAGGAATGGA GGCCTTTCCA GTCTTAATTC 9150

TGTGAGAAAT AGCTGCAAAT CGACGGTTGA GCTCAGAGAT TTGGGGCTCT 9200

ACTACTTTCC TGCAGTGGTC ACCGCGGTTT GCCATCAATT TTGCTGCTTG 9250

GTCACGTGTG GAGTCCACCT TTGGGCGCAT GTCATTCATT TCAGCCTTTA 9300

AACGCTTAAG AATGTCTTCC TTTTGTTGTG GTTTCTTCTT TTCAGACTCA 9350

TCTAAAAGTT CATCTGCATG AATGATCCAC TTTGTGATTT GTTCTATGTT 9400

CTGATCAAAG GTTTCCATGT GTTTCTGGTA TTCCAACAAA AGATTTAGCC 9450

ATTCTTCTAC TCTGGAGGTG ACAGCTATCC AGTTACTGTT CAGAAGACTC 9500

AGTTTATCTT CTACCAAGGT TTCTTTCTTG CCCAACACCA TTTTCAAAGA 9550

CTCTCCTAAT TCTGTAACAC TCTTCAAGTG AGCCTTCTGT TTCTCAATCT 9600

CTTTTTGAGT AGCCTTTCCC CAGGCAACTT CAGAATCCAA ATTACTTGGC 9650

ATTCCTTCAA CTGCTGATCT CTTCGTCAAT TC[illegible]GTATCTG TTGCTGCCAG 9700

CCATTCTGTT AAGACATTCA TTTCCTTTCT CATCTTACGG GACAACTTCA 9750

AGCATTTCTC CAACTGTTGC TTTCTCTCTG TTACCTTCGC ACCCAACTCA 9800

TTGTAATGCA ATTTCAAAGC TGTTACTCGT TCATCAAGCT CTTTGGGATT 9850

TTCTGTCTGC TTTTTCTGTA CAATTTGACG TCCGGTTTTA ATCACCATTT 9900

CCACTTCAGA CTTGACTTCA CTCAGGCTTT TATACAAGTT CACACAATGA 9950

CTTAGTTGTG ACTGAATTAC TTCCTGTTCA ACACTCTTGG TTTCCAATGC 10000

AGGCAAATGC ATCTTGACTT CATCTAAAAT CATCTTACTT TCCTCTAGAC 10050

GTTGTTCAAA ATTGGCTGGT TTTTGGAATA ATCGAAATTT CATGGAGACA 10100

TCTTGTAATT TTTTCTGTGC AACATCAATT TGTGAAAGAA CCCTTTGGTT 10150

GGCATCCTTC CCCTGGTTAT GTTTCTTCAT TTCTTCTAAA CTTATCTCAT 10200

GACTTGTCAA ATCTGATTGG ATTTTCTGGG CTTCCTGAGG CATTTGAGCT 10250

GCATCCACCT TGTCAGTGAT ATAAGCTGCC AACTGCTTGT CAATGAATTC 10300

AAGCGACTCC TGAATTAAGT GCAAGGACTT TTCAATTTCC TGGGCAGACT 10350

GGATACTCTG TTCAAGCAAC TTTTGTTTCC TCACAGCCTC TTCATGTAGT 10400

TCCCTCCAAC GAGAATTAAA CGTCTCAAGC TCCTCATTGA TCAGTTCATC 10450

CATGACTCCT CCATCTGTAA GAGTCTGTGC CAATAGACGA ATCTGATTTG 10500

GGTTCTCCTC TGAATGATGC ATCAGATTTT CAAGAGATTC TAGCACTTCA 10550

GTGATTTCCT CAGGTCCTGC AGGAACATTT TCCATGGTTT TAAGTTTCAA 10600

TTCTACTTCA TTGAGCCACT TGTTTGCTTT CTCTAAATAT GACAATAACT 10650

CATGCCAACA TGCCCAAACT TCTTCCAAAG TTTTGCATTT TCCATTCAGC 10700

CTGGTGCACA GCCATTGGTA GTTGGTGGTC AGAGTTTCAA GTTCCTTTTT 10750

TAAGGCCTCT TGTGCTGAGG GTGGAGCGTG AGCTATTACA CTATTTACAG 10800

TCTCAGTAAG GAGTTTCACT TTAGTTTCTT TTTGTAGTGC CTCTTCTTTA 10850
GCTCTCTTCA TTTCTTCAAC AGCAGTCTGT AATTCATCTG GAGTTTTATA 10900
TTCAAAATCT CTCTCTAGAT ATTCTTCTTC AGCTTGTGTC ATCCACTCAT 10950
GCATCTCTGA TAGATCTTTT TGGAGGCTTA CGGTTTTATC CAAACCTGCC 11000
TTTAAGGCTT CCTTTCTGGT GTAGACCTGG CGGCATATGT GATCCCACTG 11050
AGTGTTAAGC TCTCTAAGTT CTGTCTCCAG TCTGGATGCA AACTCAAGTT 11100
CAGCTTCACT CTTTATCTTC TGCCCACCTT CATTAACACT ATTTAAACTG 11150
GGCTGAATTG TTTGAATATC ACCAACTAAA AGTCTGCATT GTTTGAGCTG 11200
TTTTTTCAGG ATTTCAGCAT CCCCCAGGGC AGGCCATTCC TCTTTCAGGA 11250
AAACATCAAC TTCAGCCATC CATTTCTGTA AGGTTTTTAT GTGATTCTGA 11300
AATTTTCGAA GTTTATTCAT ATGTTCTTCT AGCTTTTGGC AGCTTTCCAC 11350
CAACTGGGAG GAAAGTTTCT TCCAGTGCCC CTCAATCTCT TCAAATTCTG 11400
ACAGATATTT CTGGCATATT TCTGAAGGTG CTTTCTTGGC CATCTCCTTC 11450
ACAGTGTCAC TCAGATAGTT GAAGCCATTT TGTTGCTCTT TCAAAGAACT 11500
TTGCAGAGCC TGTAATTTCC CGAGTCTCTC CTCCATTATT TCATATTCAG 11550
TAACACTAAG ATAAGGTACA GAGAGTTTGC TTTCTGACTG CTGGATCCAC 11600
GTCCTGATGC TACTCATTGT CTCCTGATAG CGCATTGGTG GTAAAGTGTC 11650
AAAAATTGTC TGTAGCTCTT TCTCTTTGGC CCTCACACCA TCAAAGATGT 11700
GGTTAAAATG ATTAGTAAAG GCCACAAAGT CTGCATCCAG AAACATTGGC 11750
CCCTGTCCCT TTTCTTTCAG TTGTAGACTC TGAATTTTTA ATTGCTCAAT 11800
TTGAGGCTGA AGAGCTGACA ATCTGTTGAC TTCATCCTTA CAAATTTTTA 11850
ACTGGCTTTT AATTGCTGTT GGCTCTGATA GGGTGGTAGA CTGGGTTTTC 11900
AACAAGTTTT CGGCAGTAGT TGTCATCTGT TCCAATTGTT GTAGCTGATT 11950
ATAAAAGGTA ATGATGTTGG TTTGATACTC TAGCCAGTTA ACTCTCTCAC 12000
TCAGCAATTG GCAGAATTCT GTCCACCGGC TGTTCAGTTG TTCTGAAGCT 12050
TGTCTGATAC TTTCAGCATT AACACCCTCA TTTGCCATCT GTTCCACCAG 12100

GGCCTGAGCT GATCTGCTGC CATCTTGCAG TTTTCTGAAC TTCTCTGCTT 12150

TTTCTCGTGC TATGGCATTG ACTTTTTCTT GCAAGTCTGA GATGTTGCCT 12200

TCTTTTCGAT AGACTGCAAA TTCAGAACTC TGTAATACAG CTTCTGAACG 12250

AGTAATCCAA CTGTGAAGTT CAGTTATATC GACATCCAAC CTTTTCCTGA 12300

GTTCAGAATC CACAGTTATC TGCCTCTTCT TTTGAGGAGG TGGTGGTGGA 12350

AGTTCCTCTT GGGCATGTTT TACCATGATT TGTTCCCTTG TGGTCACCAT 12400

AGTTACCGTT TCCATTACAG TTGTCTGTGT TAGGGATGGT TGAGTGGTGG 12450

TGACAGCCTG TGAAATTTGT GCTGAACTCT TTTCAAGTTT TTGGGTTAAA 12500

TTGTCCCAAC GTTGTGCAAA GTTTTCCATC CAGATTTCCA TCTTTTGAGT 12550

CACTGACTTA TTTTTCAGTG CCGAAAGTAG ATCTTGATTG AGTGAACTTA 12600

GTTTTTCCAT GGTTGGCTTT TTCTTTTCTA GATCTATTTT TAAAGTAGAT 12650

ATTTTGTGAA GACTTGACAT CATTTCATTT TGATCTTTAA AGCCACTTGT 12700

CTGAATGTTC TTCATTGCAT CTTCTTTTTC TGAAAGCCAT GTACTAAAAA 12750

GGCACTGTTC TTCAGTAAAA TGCTGCCATT TTAGAAGAAT ATCTTGTAAA 12800

ACAATCCAGC GGTCTTCAGT CCATCTGCAG ATATTTGCCC ATCGATCTCC 12850

CAGTACCTTA AGTTGTTCTT CCAAAGCAGC TGTTGCATGA TCACCGCTGG 12900

ATTCATCAAC CACTACTACC ATGTGAGTGA GCGAGTTGAC CCTGACCTGC 12950

TCCTGTTCTA GATCTTCTTG AAGCACCTTA TGTTGTTGTA CTTGGCATTT 13000

TAGATCTTCA AGATCAGGTC CAAAGGGCTC TTCCTCCATT TTCTTAGTTC 13050

TCTCTTCAGT TTTTGTTAAC CAGTCATCTA GTTCTTTTAA TTTCTGATTC 13100

TGGAGATCCA TTAGAACTTT GTGTAATTTG CTTTGTTTTT CCATGCTAGC 13150

TACCCTGAGA CATTCCCATC TTGAATTTAG GAGATTCATT TGTTCTTGCA 13200

CTTCAGCTTC TTCATCTTCT GATAATTTCC CTTTTCCAAC TAGTTGACTT 13250

CCTAACTGTA GAACATTACC AACAAGTCCT TGATGAGATG TCAGATCCAT 13300

CATGAATCCC TCATGAGCAT GAAACTGTTC TTTCACTTCT TCAACATCAT 13350

TTGAAATCTC TCCTTGTGCT CGCAATGTAT CCTCGGCAGA AAGAAGCCAT 13400

GAAAGTACTT CTTCTAAAGC AGTTTGGTAA CTATCCAGAT TTACTTCCGT 13450

CTCCATCAAT GAACTGTCAA GTGACTTGTC TCTGGGAGCT TCCAAATGCT 13500

GTGAAGGATA GGGGCTCTGT GTGGAATCAG AGGTGGCAAC ATAAGCAGCC 13550

TGTGTGAAGG CATAACTCTT GAATCGAGGC TAAGGAGATG AAGAAGTTTG 13600

TTCATAGCCC TGTGCTAGAC TGACTGTGAT CTGTTGAGAG TAATGCATCT 13650

GGTGATGTAA TTGAAAATGT TCTTCTCTAG TTACTTTTGA AGATGTCCTG 13700

GGCAACATTT CCACTTCTTG AATGGCTTCA ATGCTCACTT GTTGTGGCAA 13750

AACTTGAAAG AGTGATGTGA TGTACATTAA GATGGACTTC TTGTCTGGAT 13800

AAGTGGTAGC AACATCTTCA GGATCAAGAA GTTTTTCTAT GCCTAACTGG 13850

CATTTTGCAA TGTTGAAGGC ATGTTCCAGT CTTTGGGTGG CTGAGTGCTG 13900

TGAAACCACA CTATTCCAAT CAAACAGGTC GGGCCTGTGA CTATGGATAA 13950

GAGCATTCAA AGCCAACCCG TCGGACCAGC TAGAGGTGAA GTTGATGACG 14000

TTAACCTGTG GATAATTACG TGTTGACTGT CGAACCCAGC TCAGAAGAAT 14050

CTTTTCACTG TTGGTTTGCT GCAATCCAGC CATGATAGTT TTCATCACAT 14100

TTTTGACCTG CCAGTGGAGG ATTATATTCC AAATCAAACC AAGAGTGAGT 14150

TTATGATTTC CATCCACTAT GTCAGTGCTT CCTATATTCA CTAAATCAAC 14200

ATTATTTTTC TGTAAGACCC GCAGTGCCTT GTTGACATTG TTCAGGGCAT 14250

GAACTCTTGT AGATCCCTTT TCTTTTGGCA GTTTTTGCCC TGTAAGGCCT 14300

TCCAAGAGGT CTAGGAGGCG TTTTCCATCC TGCAGGTCAC TGAAGAGGTT 14350

GTCTATGTGT TGCTTTCCAA ACTTAGAAAA TTGTGCATTT ATCCATTTTG 14400

TGAATGTTTT CTTTTGAACA TCTTCTCTTT CATAACAGTC CTCTACTTCT 14450

TCCCACCAAA GCATTTGGAA GAAAAAGTAT ATATCAAGGC AGGGATAAAA 14500

ATCTTGGTAA AAGTTTCTCC CAGTTTTATT GCTCCAGGAG GCTTAGGTAC 14550

GATGAGAAGC CAATAAACTT CAGCAGCCTT GACAAAAAAA AAAAAAAAAA 14600

TAGCACTTCA AGTCTTCCTA TTCGTTTTTT CTATAAAGCT ATTGCCTTCA 14650

AGAGCGGAAT TCCTGCAGCC CGGGGGATCC ACTAGTTCTA GAGCGGCCGC 14700

GGGTACAATT	CCGCAGCTTT	TAGAGCAGAA	GTAACACTTC	CGTACAGGCC	14750
TAGAAGTAAA	GGCAACATCC	ACTGAGGAGC	AGTTCTTTGA	TTTGCACCAC	14800
CACCGGATCC	GGGACCTGAA	ATAAAAGACA	AAAAGACTAA	ACTTACCAGT	14850
TAACTTTCTG	GTTTTTCAGT	TCCTCGAGTA	CCCGATCCTC	TAGAGTCCGG	14900
AGGCTGGATC	GGTCCCGGTG	TCTTCTATGG	AGGTCAAAAC	AGCGTGGATG	14950
GCGTCTCCAG	GCGATCTGAC	GGTTCACTAA	ACGAGCTCTG	CTTATATAGA	15000
CCTCCCACCG	TACACGCCTA	CCGCCCATTT	GCGTCAATGG	GGCGGAGTTG	15050
TTACGACATT	TTGGAAAGTC	CCGTTGATTT	TGGTGCCAAA	ACAAACTCCC	15100
ATTGACGTCA	ATGGGGTGGA	GACTTGGAAA	TCCCCGTGAG	TCAAACCGCT	15150
ATCCACGCCC	ATTGATGTAC	TGCCAAAACC	GCATCACCAT	GGTAATAGCG	15200
ATGACTAATA	CGTAGATGTA	CTGCCAAGTA	GGAAAGTCCC	ATAAGGTCAT	15250
GTACTGGGCA	TAATGCCAGG	CGGGCCATTT	ACCGTCATTG	ACGTCAATAG	15300
GGGGCGTACT	TGGCATATGA	TACACTTGAT	GTACTGCCAA	GTGGGCAGTT	15350
TACCGTAAAT	ACTCCACCCA	TTGACGTCAA	TGGAAAGTCC	CTATTGGCGT	15400
TACTATGGGA	ACATACGTCA	TTATTGACGT	CAATGGGCGG	GGGTCGTTGG	15450
GCGGTCAGCC	AGGCGGGCCA	TTTACCGTAA	GTTATGTAAC	GACCTGCAGG	15500
TCGACTCTAG	AGGATCTCCC	TAGACAAATA	TTACGCGCTA	TGAGTAACAC	15550
AAAATTATTC	AGATTTCACT	TCCTCTTATT	CAGTTTTCCC	GCGAAAATGG	15600
CCAAATCTTA	CTCGGTTACG	CCCAAATTTA	CTACAACATC	CGCCTAAAAC	15650
CGCGCGAAAA	TTGTCACTTC	CTGTGTACAC	CGGCGCACAC	CAAAAACGTC	15700
ACTTTTGCCA	CATCCGTCGC	TTACATGTGT	TCCGCCACAC	TTGCAACATC	15750
ACACTTCCGC	CACACTACTA	CGTCACCCGC	CCCGTTCCCA	CGCCCCGCGC	15800
CACGTCACAA	ACTCCACCCC	CTCATTATCA	TATTGGCTTC	AATCCAAAAT	15850
AAGGTATATT	ATTGATGATG	CTAGCGGGGC	CCTATATATG	GATCCAATTG	15900
CAATGATCAT	CATGACAGAT	CTGCGCGCGA	TCGATATCAG	CGCTTTAAAT	15950
TTGCGCATGC	TAGCTATAGT	TCTAGAGGTA	CCGGTTGTTA	ACGTTAGCCG	16000

GCTACGTATA CTCCGGAATA TTAATAGGCC TAGGATGCAT ATGGCGGCCG 16050
GCCGCCTGCA GCTGGCGCCA TCGATACGCG TACGTCGCGA CCGCGGACAT 16100
GTACAGAGCT CGAGAAGTAC TAGTGGCCAC GTGGGCCGTG CACCTTAAGC 16150
TTGGCACTGG CCGTCGTTTT ACAACGTCGT GACTGGGAAA ACCCTGGCGT 16200
TACCCAACTT AATCGCCTTG CAGCACATCC CCCTTTCGCC AGCTGGCGTA 16250
ATAGCGAAGA GGCCCGCACC GATCGCCCTT CCCAACAGTT GCGCAGCCTG 16300
AATGGCGAAT GGCGCCTGAT GCGGTATTTT CTCCTTACGC ATCTGTGCGG 16350
TATTTCACAC CGCATACGTC AAAGCAACCA TAGTACGCGC CCTGTAGCGG 16400
CGCATTAAGC GCGGCGGGTG TGGTGGTTAC GCGCAGCGTG ACCGCTACAC 16450
TTGCCAGCGC CCTAGCGCCC GCTCCTTTCG CTTTCTTCCC TTCCTTTCTC 16500
GCCACGTTCG CCGGCTTTCC CCGTCAAGCT CTAAATCGGG GGCTCCCTTT 16550
AGGGTTCCGA TTTAGTGCTT TACGGCACCT CGACCCCAAA AAACTTGATT 16600
TGGGTGATGG TTCACGTAGT GGGCCATCGC CCTGATAGAC GGTTTTTCGC 16650
CCTTTGACGT TGGAGTCCAC GTTCTTTAAT AGTGGACTCT TGTTCCAAAC 16700
TGGAACAACA CTCAACCCTA TCTCGGGCTA TTCTTTTGAT TTATAAGGGA 16750
TTTTGCCGAT TTCGGCCTAT TGGTTAAAAA ATGAGCTGAT TTAACAAAAA 16800
TTTAACGCGA ATTTTAACAA AATATTAACG TTTACAATTT TATGGTGCAC 16850
TCTCAGTACA ATCTGCTCTG ATGCCGCATA GTTAAGCCAG CCCCGACACC 16900
CGCCAACACC CGCTGACGCG CCCTGACGGG CTTGTCTGCT CCCGGCATCC 16950
GCTTACAGAC AAGCTGTGAC CGTCTCCGGG AGCTGCATGT GTCAGAGGTT 17000
TTCACCGTCA TCACCGAAAC GCGCGAGACG AAAGGGCCTC GTGATACGCC 17050
TATTTTTATA GGTTAATGTC ATGATAATAA TGGTTTCTTA GACGTCAGGT 17100
GGCACTTTTC GGGGAAATGT GCGCGGAACC CCTATTTGTT TATTTTTCTA 17150
AATACATTCA AATATGTATC CGCTCATGAG ACAATAACCC TGATAAATGC 17200
TTCAATAATA TTGAAAAAGG AAGAGTATGA GTATTCAACA TTTCCGTGTC 17250
GCCCTTATTC CCTTTTTTGC GGCATTTTGC CTTCCTGTTT TTGCTCACCC 17300

AGAAACGCTG GTGAAAGTAA AAGATGCTGA AGATCAGTTG GGTGCACGAG 17350
TGGGTTACAT CGAACTGGAT CTCAACAGCG GTAAGATCCT TGAGAGTTTT 17400
CGCCCCGAAG AACGTTTTCC AATGATGAGC ACTTTTAAAG TTCTGCTATG 17450
TGGCGCGGTA TTATCCCGTA TTGACGCCGG GCAAGAGCAA CTCGGTCGCC 17500
GCATACACTA TTCTCAGAAT GACTTGGTTG AGTACTCACC AGTCACAGAA 17550
AAGCATCTTA CGGATGGCAT GACAGTAAGA GAATTATGCA GTGCTGCCAT 17600
AACCATGAGT GATAACACTG CGGCCAACTT ACTTCTGACA ACGATCGGAG 17650
GACCGAAGGA GCTAACCGCT TTTTTGCACA ACATGGGGGA TCATGTAACT 17700
CGCCTTGATC GTTGGGAACC GGAGCTGAAT GAAGCCATAC CAAACGACGA 17750
GCGTGACACC ACGATGCCTG TAGCAATGGC AACAACGTTG CGCAAACTAT 17800
TAACTGGCGA ACTACTTACT CTAGCTTCCC GGCAACAATT AATAGACTGG 17850
ATGGAGGCGG ATAAAGTTGC AGGACCACTT CTGCGCTCGG CCCTTCCGGC 17900
TGGCTGGTTT ATTGCTGATA AATCTGGAGC CGGTGAGCGT GGGTCTCGCG 17950
GTATCATTGC AGCACTGGGG CCAGATGGTA AGCCCTCCCG TATCGTAGTT 18000
ATCTACACGA CGGGGAGTCA GGCAACTATG GATGAACGAA ATAGACAGAT 18050
CGCTGAGATA GGTGCCTCAC TGATTAAGCA TTGGTAACTG TCAGACCAAG 18100
TTTACTCATA TATACTTTAG ATTGATTTAA AACTTCATTT TTAATTTAAA 18150
AGGATCTAGG TGAAGATCCT TTTTGATAAT CTCATGACCA AAATCCCTTA 18200
ACGTGAGTTT TCGTTCCACT GAGCGTCAGA CCCCGTAGAA AAGATCAAAG 18250
GATCTTCTTG AGATCCTTTT TTTCTGCGCG TAATCTGCTG CTTGCAAACA 18300
AAAAAACCAC CGCTACCAGC GGTGGTTTGT TTGCCGGATC AAGAGCTACC 18350
AACTCTTTTT CCGAAGGTAA CTGGCTTCAG CAGAGCGCAG ATACCAAATA 18400
CTGTTCTTCT AGTGTAGCCG TAGTTAGGCC ACCACTTCAA GAACTCTGTA 18450
GCACCGCCTA CATACCTCGC TCTGCTAATC CTGTTACCAG TGGCTGCTGC 18500
CAGTGGCGAT AAGTCGTGTC TTACCGGGTT GGACTCAAGA CGATAGTTAC 18550
CGGATAAGGC GCAGCGGTCG GGCTGAACGG GGGGTTCGTG CACACAGCCC 18600

AGCTTGGAGC GAACGACCTA CACCGAACTG AGATACCTAC AGCGTGAGCT 18650
ATGAGAAAGC GCCACGCTTC CCGAAGGGAG AAAGGCGGAC AGGTATCCGG 18700
TAAGCGGCAG GGTCGGAACA GGAGAGCGCA CGAGGGAGCT TCCAGGGGGA 18750
AACGCCTGGT ATCTTTATAG TCCTGTCGGG TTTCGCCACC TCTGACTTGA 18800
GCGTCGATTT TTGTGATGCT CGTCAGGGGG GCGGAGCCTA TGGAAAAACG 18850
CCAGCAACGC GGCCTTTTTA CGGTTCCTGG CCTTTTGCTG GCCTTTTGCT 18900
CACATGTTCT TTCCTGCGTT ATCCCCTGAT TCTGTGGATA ACCGTATTAC 18950
CGCCTTTGAG TGAGCTGATA CCGCTCGCCG CAGCCGAACG ACCGAGCGCA 19000
GCGAGTCAGT GAGCGAGGAA GCGGAAGAGC GCCCAATACG CAAACCGCCT 19050
CTCCCCGCGC GTTGGCCGAT TCATTAATGC AGCTGGCACG ACAGGTTTCC 19100
CGACTGGAAA GCGGGCAGTG AGCGCAACGC AATTAATGTG AGTTAGCTCA 19150
CTCATTAGGC ACCCCAGGCT TTACACTTTA TGCTTCCGGC TCGTATGTTG 19200
TGTGGAATTG TGAGCGGATA ACAATTTCAC ACAGGAAACA GCTATGACCA 19250
TGATTACGAA TTCGAATGGC CATGGGACGT CGACCTGAGG TAATTATAAC 19300
CCGGGCC 19307

WHAT IS CLAIMED IS:

1. A recombinant shuttle vector comprising:

(a) the DNA sequences of, or corresponding to, a portion of the genome of an adenovirus which comprises DNA sequences of, or corresponding to, the adenovirus 5' and 3' inverted terminal repeats and packaging/enhancer domain necessary for replication and virion encapsidation in the absence of sequence encoding viral genes;

(b) a selected gene operatively linked to regulatory sequences directing its expression, said gene operatively linked to the DNA of (a) and capable of expression in a target cell *in vivo* or *in vitro*.

2. The vector according to claim 1 wherein said DNA sequences (a) comprise the native adenovirus 5' inverted terminal repeats and packaging sequences.

3. The vector according to claim 1 wherein said DNA sequences (a) comprise the native adenovirus 3' inverted terminal repeat sequences.

4. The vector according to claim 1 wherein said selected gene (b) is a reporter gene.

5. The vector according to claim 4 wherein said reporter gene is selected from the group consisting of the genes encoding β-galactosidase, alkaline phosphatase and green fluorescent protein.

6. The vector according to claim 1 wherein said selected gene (b) is a therapeutic gene.

7. The vector according to claim 6 wherein said therapeutic gene is selected from the group consisting of a normal CFTR gene, a DMD Becker allele and a normal LDL gene.

8. A crippled adenovirus helper virus comprising a modified adenovirus sequence in place of native adenovirus sequence map units 0-1, which modification reduces the packaging efficiency of said virus, said virus also containing selected adenovirus genes necessary to direct a productive viral infection.

9. The helper virus according to claim 8 wherein said modified sequence comprises:

i. a fragment of adenovirus map units 0-1;

ii. a fragment of (i) containing a 5' inverted terminal repeat and between one to four selected packaging sequences,

iii. a modified fragment of (i) containing at least one PAC consensus sequence in place of at least one native PAC sequence; and

iv. a modified fragment of (ii), wherein said native PAC sequences are mutated to contain modified sequences.

10. The virus according to claim 8 wherein said modified sequence comprises Ad5 base pairs 1-269.

11. The virus according to claim 8 wherein said sequence (ii) comprises Ad5 base pairs 1-321.

12. The virus according to claim 8 wherein said helper adenovirus is conjugated to a poly-cation sequence.

13. A method for producing a recombinant adenovirus which comprises transfecting a selected host cell with

(a) a recombinant shuttle vector comprising

i. the DNA sequences of, or corresponding to, a portion of the genome of an adenovirus which comprises adenovirus 5' and 3' cis-elements necessary for replication and virion encapsidation in the absence of sequence encoding viral genes; and

ii. a selected gene operatively linked to regulatory sequences directing its expression, said gene linked to the DNA of (a) and capable of expression in a target cell *in vivo* or *in vitro*; and

(b) a helper adenovirus comprising sufficient adenovirus gene sequences necessary for a productive viral infection, wherein said transfected host cell permits the formation of a recombinant virus comprising the DNA of (i) and (ii) in an adenoviral capsid, and

isolating and purifying the recombinant virus from said cell.

14. The method according to claim 13, wherein said helper virus is a crippled helper virus comprising a modified adenovirus sequence in place of native adenovirus sequence map units 0-1, which modification reduces the packaging efficiency of said helper virus, said helper virus also containing selected adenovirus genes necessary to direct a productive viral infection.

15. The method according to claim 13 wherein said helper adenovirus is associated with a poly-cation sequence.

16. The method according to claim 13 wherein said vector is associated with said helper adenovirus conjugate in a single particle.

17. The method according to claim 13 wherein said helper virus is an adenovirus sequence containing deletions of all or portions of the E1a and E1b genes.

18. The method according to claim 13 wherein said helper virus is an adenovirus sequence containing deletions of all or a portion of the E3 gene.

19. A recombinant adenovirus comprising

i. the DNA of, or corresponding to, a portion of the genome of an adenovirus which comprises adenovirus 5' and 3'cis-elements necessary for replication and virion encapsidation in the absence of sequence encoding viral genes;

ii. a selected gene operatively linked to regulatory sequences directing its expression, said gene linked to the DNA of (a) and capable of expression in a target cell *in vivo* or *in vitro;*

said DNA and gene encapsidated in an adenoviral capsid.

20. The virus according to claim 19 wherein said viral capsid is a capsid of an adenovirus serotype selected from the group consisting of types 2, 4, 5, 7, 12 and 40.

21. The virus according to claim 19 wherein said selected gene is a CFTR gene, a DMD gene and an LDL gene.

22. The use of a recombinant adenovirus according to claim 19 for the manufacture of a pharmaceutical composition suitable for delivering and integrating a selected gene into the chromosome of a target cell.

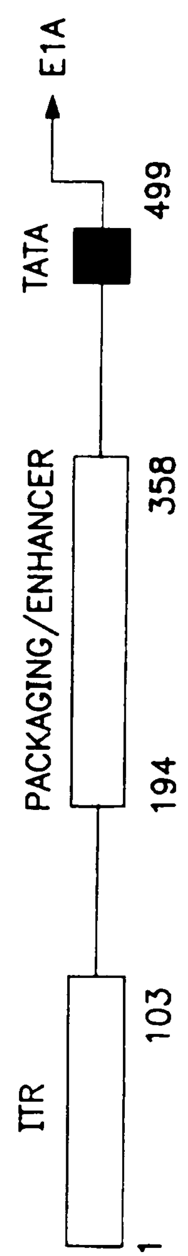
ITR
1
103
194
PACKAGING/ENHANCER
358
TATA
499
E1A

FIG. 1A

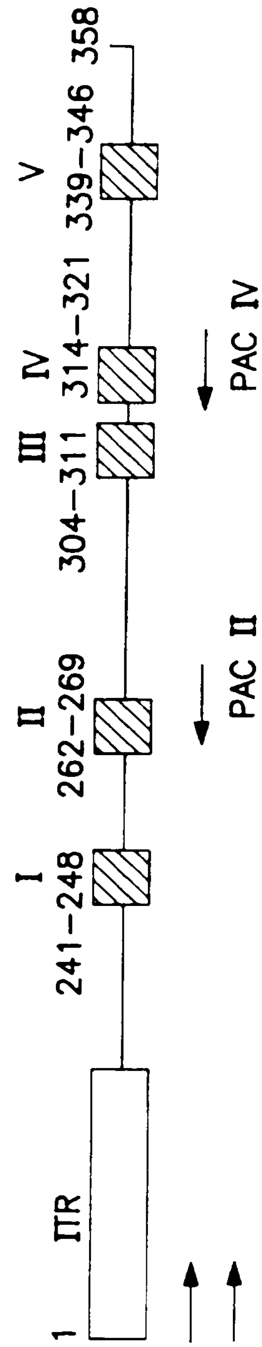
ITR
1
I
241–248
II
262–269
PAC II
304–311
III
IV
314–321
PAC IV
V
339–346
358

FIG. 1B

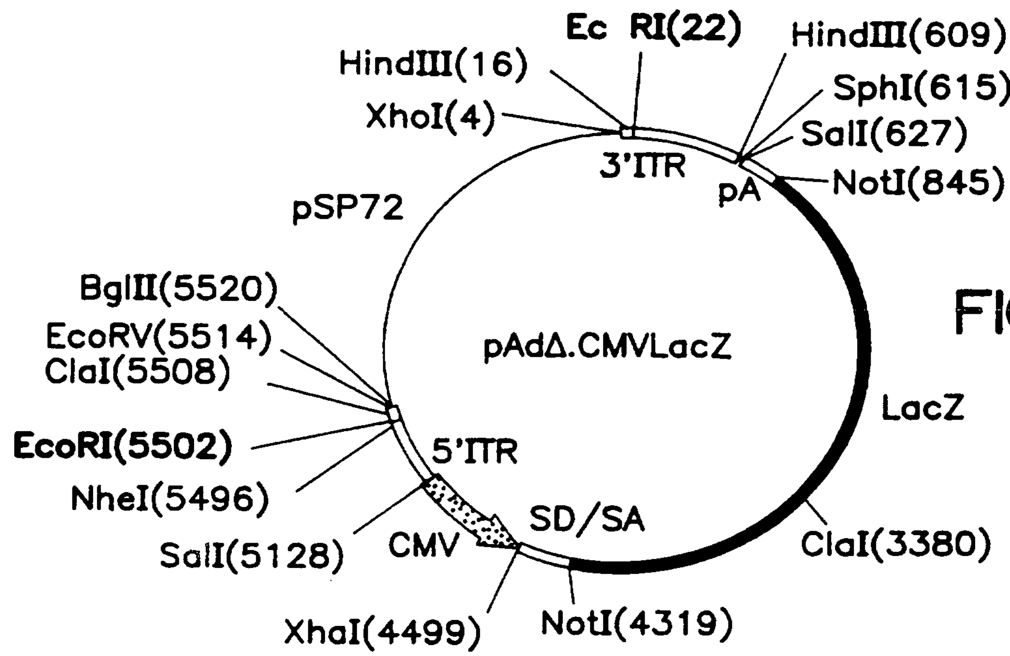

FIG. 2A

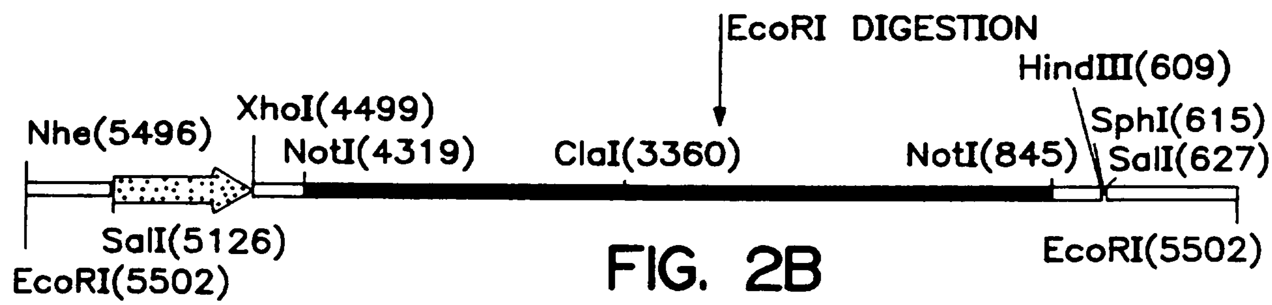

FIG. 2B

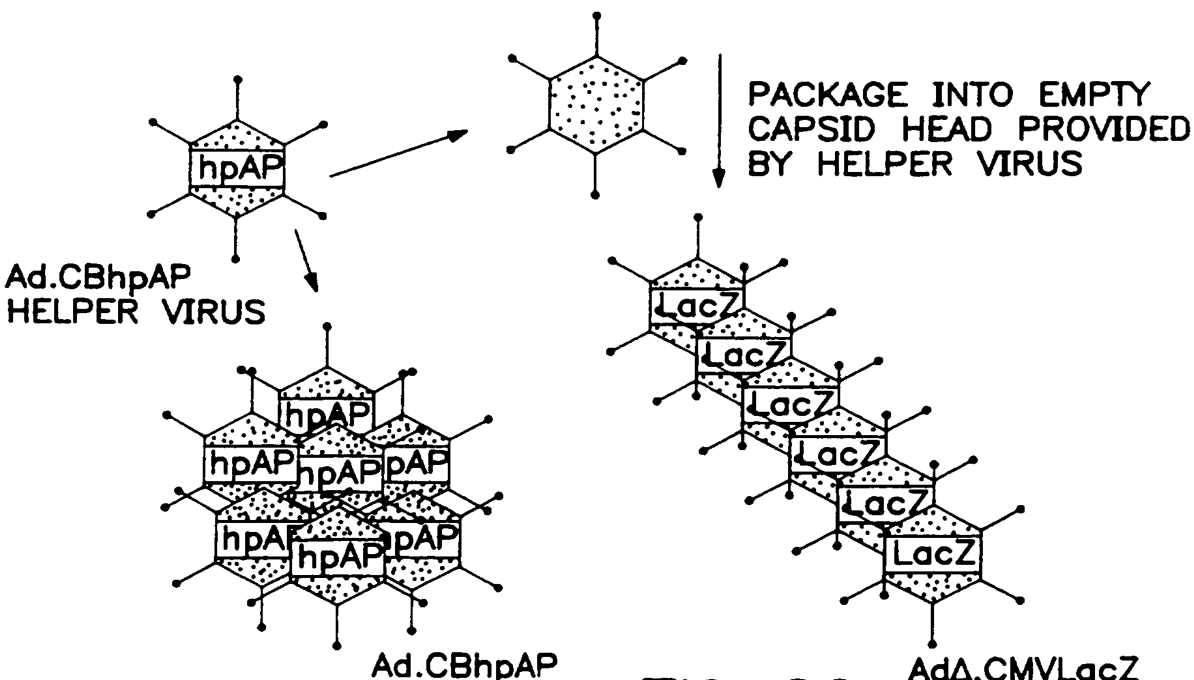

FIG. 2C

FIGURE 3A

```
GAACTCGAGC AGCTGAAGCT TGAATTCCAT CATCAATAAT ATACCTTATT     50
TTGGATTGAA GCCAATATGA TAATGAGGGG GTGGAGTTTG TGACGTGGCG    100
CGGGGCGTGG GAACGGGGCG GGTGACGTAG GTTTTAGGGC GGAGTAACTT    150
GTATGTGTTG GGAATTGTAG TTTTCTTAAA ATGGGAAGTT ACGTAACGTG    200
GGAAAACGGA AGTGACGATT TGAGGAAGTT GTGGGTTTTT TGGCTTTCGT    250
TTCTGGGCGT AGGTTCGCGT GCGGTTTTCT GGGTGTTTTT TGTGGACTTT    300
AACCGTTACG TCATTTTTTA GTCCTATATA TACTCGCTCT GCACTTGGCC    350
CTTTTTTACA CTGTGACTGA TTGAGCTGGT GCCGTGTCGA GTGGTGTTTT    400
TTTAATAGGT TTTCTTTTTT ACTGGTAAGG CTGACTGTTA GGCTGCCGCT    450
GTGAAGCGCT GTATGTTGTT CTGGAGCGGG AGGGTGCTAT TTTGCCTAGG    500
CAGGAGGGTT TTTCAGGTGT TTATGTGTTT TTCTCTCCTA TTAATTTTGT    550
TATACCTCCT ATGGGGGCTG TAATGTTGTC TCTACGCCTG CGGGTATGTA    600
TTCCCCCCAA GCTTGCATGC CTGCAGGTCG ACTCTAGAGG ATCCGAAAAA    650
ACCTCCCACA CCTCCCCCTG AACCTGAAAC ATAAAATGAA TGCAATTGTT    700
GTTGTTAACT TGTTTATTGC AGCTTATAAT GGTTACAAAT AAAGCAATAG    750
CATCACAAAT TTCACAAATA AAGCATTTTT TTCACTGCAT TCTAGTTGTG    800
GTTTGTCCAA ACTCATCAAT GTATCTTATC ATGTCTGGAT CCCCGCGGCC    850
GCCTAGAGTC GAGGCCGAGT TTGTCAGAAA GCAGACCAAA CAGCGGTTGG    900
AATAATAGCG AGAACAGAGA AATAGCGGCA AAAATAATAC CCGTATCACT    950
TTTGCTGATA TGGTTGATGT CATGTAGCCA AATCGGGAAA AACGGGAAGT   1000
AGGCTCCCAT GATAAAAAAG TAAAAGAAAA AGAATAAACC GAACATCCAA   1050
AAGTTTGTGT TTTTTAAATA GTACATAATG GATTTCCTTA CGCGAAATAC   1100
GGGCAGACAT GGCCTGCCCG GTTATTATTA TTTTTGACAC CAGACCAACT   1150
GGTAATGGTA GCGACCGGCG CTCAGCTGTA ATTCCGCCGA TACTGACGGG   1200
CTCCAGGAGT CGTCGCCACC AATCCCCATA TGGAAACCGT CGATATTCAG   1250
CCATGTGCCT TCTTCCGCGT GCAGCAGATG GCGATGGCTG CTTTCCATCA   1300
GTTGCTGTTG ACTGTAGCGG CTGATGTTGA ACTGGAAGTC GCCGCGCCAC   1350
```

FIGURE 3B

TGGTGTGGGC CATAATTCAA TTCGCGCGTC CCGCAGCGCA GACCGTTTTC 1400

GCTCGGGAAG ACGTACGGGG TATACATGTC TGACAATGGC AGATCCCAGC 1450

GGTCAAAACA GGCGGCAGTA AGGCGGTCGG GATAGTTTTC TTGCGGCCCT 1500

AATCCGAGCC AGTTTACCCG CTCTGCTACC TGCGCCAGCT GGCAGTTCAG 1550

GCCAATCCGC GCCGGATGCG GTGTATCGCT CGCCACTTCA ACATCAACGG 1600

TAATCGCCAT TTGACCACTA CCATCAATCC GGTAGGTTTT CCGGCTGATA 1650

AATAAGGTTT TCCCCTGATG CTGCCACGCG TGAGCGGTCG TAATCAGCAC 1700

CGCATCAGCA AGTGTATCTG CCGTGCACTG CAACAACGCT GCTTCGGCCT 1750

GGTAATGGCC CGCCGCCTTC CAGCGTTCGA CCCAGGCGTT AGGGTCAATG 1800

CGGGTCGCTT CACTTACGCC AATGTCGTTA TCCAGCGGTG CACGGGTGAA 1850

CTGATCGCGC AGCGGCGTCA GCAGTTGTTT TTTATCGCCA ATCCACATCT 1900

GTGAAAGAAA GCCTGACTGG CGGTTAAATT GCCAACGCTT ATTACCCAGC 1950

TCGATGCAAA AATCCATTTC GCTGGTGGTC AGATGCGGGA TGGCGTGGGA 2000

CGCGGCGGGG AGCGTCACAC TGAGGTTTTC CGCCAGACGC CACTGCTGCC 2050

AGGCGCTGAT GTGCCCGGCT TCTGACCATG CGGTCGCGTT CGGTTGCACT 2100

ACGCGTACTG TGAGCCAGAG TTGCCCGGCG CTCTCCGGCT GCGGTAGTTC 2150

AGGCAGTTCA ATCAACTGTT TACCTTGTGG AGCGACATCC AGAGGCACTT 2200

CACCGCTTGC CAGCGGCTTA CCATCCAGCG CCACCATCCA GTGCAGGAGC 2250

TCGTTATCGC TATGACGGAA CAGGTATTCG CTGGTCACTT CGATGGTTTG 2300

CCCGGATAAA CGGAACTGGA AAAACTGCTG CTGGTGTTTT GCTTCCGTCA 2350

GCGCTGGATG CGGCGTGCGG TCGGCAAAGA CCAGACCGTT CATACAGAAC 2400

TGGCGATCGT TCGGCGTATC GCCAAAATCA CCGCCGTAAG CCGACCACGG 2450

GTTGCCGTTT TCATCATATT TAATCAGCGA CTGATCCACC CAGTCCCAGA 2500

CGAAGCCGCC CTGTAAACGG GGATACTGAC GAAACGCCTG CCAGTATTTA 2550

GCGAAACCGC CAAGACTGTT ACCCATCGCG TGGGCGTATT CGCAAAGGAT 2600

CAGCGGGCGC GTCTCTCCAG GTAGCGAAAG CCATTTTTTG ATGGACCATT 2650

FIGURE 3C

TCGGCACAGC CGGGAAGGGC TGGTCTTCAT CCACGCGCGC GTACATCGGG 2700
CAAATAATAT CGGTGGCCGT GGTGTCGGCT CCGCCGCCTT CATACTGCAC 2750
CGGGCGGGAA GGATCGACAG ATTTGATCCA GCGATACAGC GCGTCGTGAT 2800
TAGCGCCGTG GCCTGATTCA TTCCCCAGCG ACCAGATGAT CACACTCGGG 2850
TGATTACGAT CGCGCTGCAC CATTCGCGTT ACGCGTTCGC TCATCGCCGG 2900
TAGCCAGCGC GGATCATCGG TCAGACGATT CATTGGCACC ATGCCGTGGG 2950
TTTCAATATT GGCTTCATCC ACCACATACA GGCCGTAGCG GTCGCACAGC 3000
GTGTACCACA GCGGATGGTT CGGATAATGC GAACAGCGCA CGGCGTTAAA 3050
GTTGTTCTGC TTCATCAGCA GGATATCCTG CACCATCGTC TGCTCATCCA 3100
TGACCTGACC ATGCAGAGGA TGATGCTCGT GACGGTTAAC GCCTCGAATC 3150
AGCAACGGCT TGCCGTTCAG CAGCAGCAGA CCATTTTCAA TCCGCACCTC 3200
GCGGAAACCG ACATCGCAGG CTTCTGCTTC AATCAGCGTG CCGTCGGCGG 3250
TGTGCAGTTC AACCACCGCA CGATAGAGAT TCGGGATTTC GGCGCTCCAC 3300
AGTTTCGGGT TTTCGACGTT CAGACGTAGT GTGACGCGAT CGGCATAACC 3350
ACCACGCTCA TCGATAATTT CACCGCCGAA AGGCGCGGTG CCGCTGGCGA 3400
CCTGCGTTTC ACCCTGCCAT AAAGAAACTG TTACCCGTAG GTAGTCACGC 3450
AACTCGCCGC ACATCTGAAC TTCAGCCTCC AGTACAGCGC GGCTGAAATC 3500
ATCATTAAAG CGAGTGGCAA CATGGAAATC GCTGATTTGT GTAGTCGGTT 3550
TATGCAGCAA CGAGACGTCA CGGAAAATGC CGCTCATCCG CCACATATCC 3600
TGATCTTCCA GATAACTGCC GTCACTCCAA CGCAGCACCA TCACCGCGAG 3650
GCGGTTTTCT CCGGCGCGTA AAAATGCGCT CAGGTCAAAT TCAGACGGCA 3700
AACGACTGTC CTGGCCGTAA CCGACCCAGC GCCCGTTGCA CCACAGATGA 3750
AACGCCGAGT TAACGCCATC AAAAATAATT CGCGTCTGGC CTTCCTGTAG 3800
CCAGCTTTCA TCAACATTAA ATGTGAGCGA GTAACAACCC GTCGGATTCT 3850
CCGTGGGAAC AAACGGCGGA TTGACCGTAA TGGGATAGGT TACGTTGGTG 3900
TAGATGGGCG CATCGTAACC GTGCATCTGC CAGTTTGAGG GGACGACGAC 3950

FIGURE 3D

AGTATCGGCC TCAGGAAGAT CGCACTCCAG CCAGCTTTCC GGCACCGCTT 4000
CTGGTGCCGG AAACCAGGCA AAGCGCCATT CGCCATTCAG GCTGCGCAAC 4050
TGTTGGGAAG GGCGATCGGT GCGGGCCTCT TCGCTATTAC GCCAGCTGGC 4100
CAAAGGGGGA TGTGCTGCAA GGCGATTAAG TTGGGTAACG CCAGGGTTTT 4150
CCCAGTCACG ACGTTGTAAA ACGACGGGAT CGCGCTTGAG CAGCTCCTTG 4200
CTGGTGTCCA GACCAATGCC TCCCAGACCG GCAACGAAAA TCACGTTCTT 4250
GTTGGTCAAA GTAAACGACA TGGTGACTTC TTTTTTGCTT TAGCAGGCTC 4300
TTTCGATCCC CGGGAATTGC GGCCGCGGGT ACAATTCCGC AGCTTTTAGA 4350
GCAGAAGTAA CACTTCCGTA CAGGCCTAGA AGTAAAGGCA ACATCCACTG 4400
AGGAGCAGTT CTTTGATTTG CACCACCACC GGATCCGGGA CCTGAAATAA 4450
AAGACAAAAA GACTAAACTT ACCAGTTAAC TTTCTGGTTT TTCAGTTCCT 4500
CGAGTACCGG ATCCTCTAGA GTCCGGAGGC TGGATCGGTC CCGGTCTCTT 4550
CTATGGAGGT CAAAACAGCG TGGATGGCGT CTCCAGGCGA TCTGACGGTT 4600
CACTAAACGA GCTCTGCTTA TATAGACCTC CCACCGTACA CGCCTACCGC 4650
CCATTTGCGT CAATGGGGCG GAGTTGTTAC GACATTTTGG AAAGTCCCGT 4700
TGATTTTGGT GCCAAAACAA ACTCCCATTG ACGTCAATGG GGTGGAGACT 4750
TGGAAATCCC CGTGAGTCAA ACCGCTATCC ACGCCCATTG ATGTACTGCC 4800
AAAACCGCAT CACCATGGTA ATAGCGATGA CTAATACGTA GATGTACTGC 4850
CAAGTAGGAA AGTCCCATAA GGTCATGTAC TGGGCATAAT GCCAGGCGGG 4900
CCATTTACCG TCATTGACGT CAATAGGGGG CGTACTTGGC ATATGATACA 4950
CTTGATGTAC TGCCAAGTGG GCAGTTTACC GTAAATACTC CACCCATTGA 5000
CGTCAATGGA AAGTCCCTAT TGGCGTTACT ATGGGAACAT ACGTCATTAT 5050
TGACGTCAAT GGGCGGGGGT CGTTGGGCGG TCAGCCAGGC GGGCCATTTA 5100
CCGTAAGTTA TGTAACGACC TGCAGGTCGA CTCTAGAGGA TCTCCCTAGA 5150
CAAATATTAC GCGCTATGAG TAACACAAAA TTATTCAGAT TTCACTTCCT 5200
CTTATTCAGT TTTCCCGCGA AAATGGCCAA ATCTTACTCG GTTACGCCCA 5250

FIGURE 3E

AATTTACTAC	AACATCCGCC	TAAAACCGCG	CGAAAATTGT	CACTTCCTGT	5300
GTACACCGGC	GCACACCAAA	AACGTCACTT	TTGCCACATC	CGTCGCTTAC	5350
ATGTGTTCCG	CCACACTTGC	AACATCACAC	TTCCGCCACA	CTACTACGTC	5400
ACCCGCCCCG	TTCCCACGCC	CCGCGCCACG	TCACAAACTC	CACCCCCTCA	5450
TTATCATATT	GGCTTCAATC	CAAAATAAGG	TATATTATTG	ATGATGCTAG	5500
CGAATTCATC	GATGATATCA	GATCTGCCGG	TCTCCCTATA	GTGAGTCGTA	5550
TTAATTTCGA	TAAGCCAGGT	TAACCTGCAT	TAATGAATCG	GCCAACGCGC	5600
GGGGAGAGGC	GGTTTGCGTA	TTGGGCGCTC	TTCCGCTTCC	TCGCTCACTG	5650
ACTCGCTGCG	CTCGGTCGTT	CGGCTGCGGC	GAGCGGTATC	AGCTCACTCA	5700
AAGGCGGTAA	TACGGTTATC	CACAGAATCA	GGGGATAACG	CAGGAAAGAA	5750
CATGTGAGCA	AAAGGCCAGC	AAAAGGCCAG	GAACCGTAAA	AAGGCCGCGT	5800
TGCTGGCGTT	TTTCCATAGG	CTCCGCCCCC	CTGACGAGCA	TCACAAAAAT	5850
CGACGCTCAA	GTCAGAGGTG	GCGAAACCCG	ACAGGACTAT	AAAGATACCA	5900
GGCGTTTCCC	CCTGGAAGCT	CCCTCGTGCG	CTCTCCTGTT	CCGACCCTGC	5950
CGCTTACCGG	ATACCTGTCC	GCCTTTCTCC	CTTCGGGAAG	CGTGGCGCTT	6000
TCTCAATGCT	CACGCTGTAG	GTATCTCAGT	TCGGTGTAGG	TCGTTCGCTC	6050
CAAGCTGGGC	TGTGTGCACG	AACCCCCCGT	TCAGCCCGAC	CGCTGCGCCT	6100
TATCCGGTAA	CTATCGTCTT	GAGTCCAACC	CGGTAAGACA	CGACTTATCG	6150
CCACTGGCAG	CAGCCACTGG	TAACAGGATT	AGCAGAGCGA	GGTATGTAGG	6200
CGGTGCTACA	GAGTTCTTGA	AGTGGTGGCC	TAACTACGGC	TACACTAGAA	6250
GGACAGTATT	TGGTATCTGC	GCTCTGCTGA	AGCCAGTTAC	CTTCGGAAAA	6300
AGAGTTGGTA	GCTCTTGATC	CGGCAAACAA	ACCACCGCTG	CTAGCGGTGG	6350
TTTTTTTGTT	TGCAAGCAGC	AGATTACGCG	CAGAAAAAAA	GGATCTCAAG	6400
AAGATCCTTT	GATCTTTTCT	ACGGGGTCTG	ACGCTCAGTG	GAACGAAAAC	6450
TCACGTTAAG	GGATTTTGGT	CATGAGATTA	TCAAAAAGGA	TCTTCACCTA	6500
GATCCTTTTA	AATTAAAAAT	GAAGTTTTAA	ATCAATCTAA	AGTATATATG	6550

FIGURE 3F

AGTAAACTTG GTCTGACAGT TACCAATGCT TAATCAGTGA GGCACCTATC 6600
TCAGCGATCT GTCTATTTCG TTCATCCATA GTTGCCTGAC TCCCCGTCGT 6650
GTAGATAACT ACGATACGGG AGGGCTTACC ATCTGGCCCC AGTGCTGCAA 6700
TGATACCGCG AGACCCACGC TCACCGGCTC CAGATTTATC AGCAATAAAC 6750
CAGCCAGCCG GAAGGGCCGA GCGCAGAAGT GGTCCTGCAA CTTTATCCGC 6800
CTCCATCCAG TCTATTAATT GTTGCCGGGA AGCTAGAGTA AGTAGTTCGC 6850
CAGTTAATAG TTTGCGCAAC GTTGTTGCCA TTGCTACAGG CATCGTGGTG 6900
TCACGCTCGT CGTTTGGTAT GGCTTCATTC AGCTCCGGTT CCCAACGATC 6950
AAGGCGAGTT ACATGATCCC CCATGTTGTG CAAAAAAGCG GTTAGCTCCT 7000
TCGGTCCTCC GATCGTTGTC AGAAGTAAGT TGGCCGCAGT GTTATCACTC 7050
ATGGTTATGG CAGCACTGCA TAATTCTCTT ACTGTCATGC CATCCGTAAG 7100
ATGCTTTTCT GTGACTGGTG AGTACTCAAC CAAGTCATTC TGAGAATAGT 7150
GTATGCGGCG ACCGAGTTGC TCTTGCCCGG CGTCAATACG GGATAATACC 7200
GCGCCACATA GCAGAACTTT AAAAGTGCTC ATCATTGGAA AACGTTCTTC 7250
GGGGCGAAAA CTCTCAAGGA TCTTACCGCT GTTGAGATCC AGTTCGATGT 7300
AACCCACTCG TGCACCCAAC TGATCTTCAG CATCTTTTAC TTTCACCAGC 7350
GTTTCTGGGT GAGCAAAAAC AGGAAGGCAA AATGCCGCAA AAAAGGGAAT 7400
AAGGGCGACA CGGAAATGTT GAATACTCAT ACTCTTCCTT TTTCAATATT 7450
ATTGAAGCAT TTATCAGGGT TATTGTCTCA TGAGCGGATA CATATTTGAA 7500
TGTATTTAGA AAAATAAACA AATAGGGGTT CCGCGCACAT TTCCCCGAAA 7550
AGTGCCACCT GACGTCTAAG AAACCATTAT TATCATGACA TTAACCTATA 7600
AAAATAGGCG TATCACGAGG CCCTTTCGTC TCGCGCGTTT CGGTGATGAC 7650
GGTGAAAACC TCTGACACAT GCAGCTCCCG GAGACGGTCA CAGCTTGTCT 7700
GTAAGCGGAT GCCGGGAGCA GACAAGCCCG TCAGGGCGCG TCAGCGGGTG 7750
TTGGCGGGTG TCGGGGCTGG CTTAACTATG CGGCATCAGA GCAGATTGTA 7800
CTGAGAGTGC ACCATATGGA CATATTGTCG TTAGAACGCG GCTACAATTA 7850
ATACATAACC TTATGTATCA TACACATACG ATTTAGGTGA CACTATA 7897

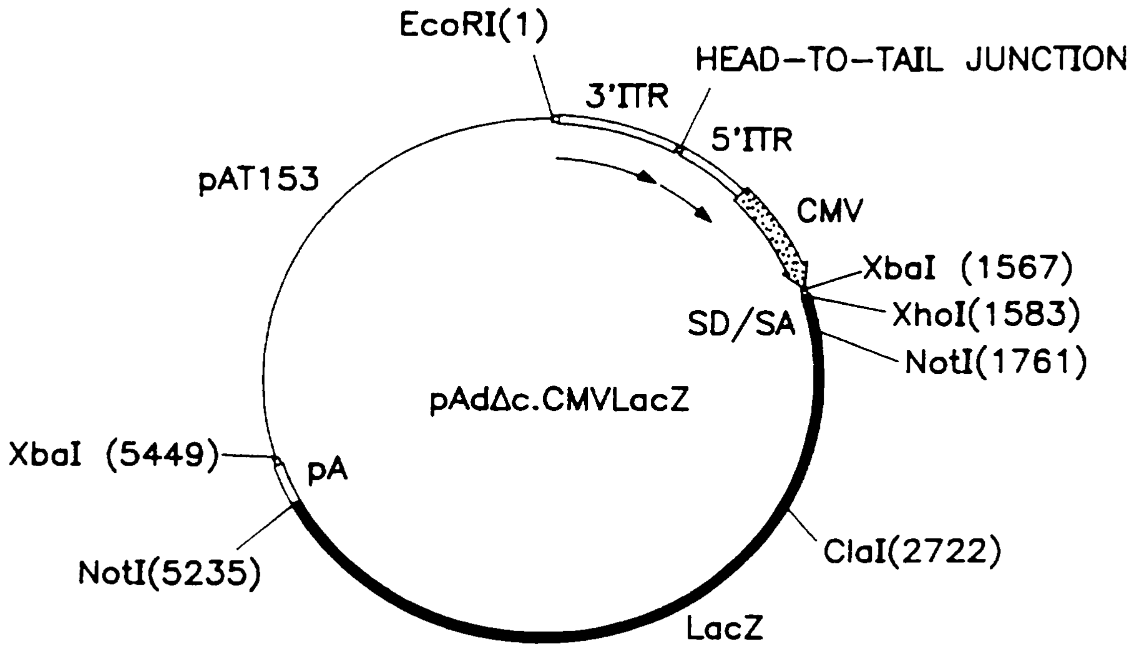
EcoRI(1)
HEAD-TO-TAIL JUNCTION
3'ITR
5'ITR
CMV
pAT153
XbaI (1567)
XhoI(1583)
SD/SA
NotI(1761)
pAdΔc.CMVLacZ
XbaI (5449)
pA
NotI(5235)
ClaI(2722)
LacZ

FIG. 4A

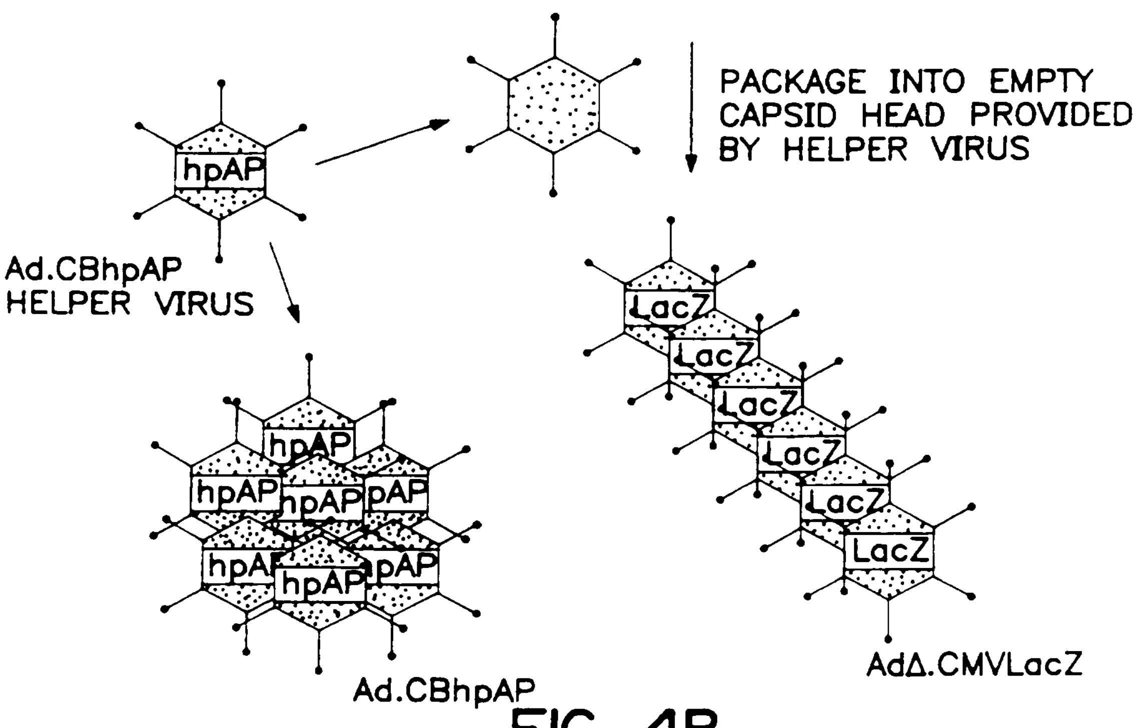
PACKAGE INTO EMPTY CAPSID HEAD PROVIDED BY HELPER VIRUS
hpAP
Ad.CBhpAP HELPER VIRUS
LacZ
hpAP
pAP
Ad.CBhpAP
AdΔ.CMVLacZ

FIG. 4B

FIGURE 5A

GAATTCGCTA GCTAGCGGGG GAATACATAC CCGCAGGCGT AGAGACAACA 50
TTACAGCCCC CATAGGAGGT ATAACAAAAT TAATAGGAGA GAAAAACACA 100
TAAACACCTG AAAAACCCTC CTGCCTAGGC AAAATAGCAC CCTCCCGCTC 150
CAGAACAACA TACAGCGCTT CACAGCGGCA GCCTAACAGT CAGCCTTACC 200
AGTAAAAAAG AAAACCTATT AAAAAAACAC CACTCGACAC GGCACCAGCT 250
CAATCAGTCA CAGTGTAAAA AAGGGCCAAG TGCAGAGCGA GTATATATAG 300
GACTAAAAAA TGACGTAACG GTTAAAGTCC ACAAAAAACA CCCAGAAAAC 350
CGCACGCGAA CCTACGCCCA GAAACGAAAG CCAAAAAACC CACAACTTCC 400
TCAAATCGTC ACTTCCGTTT TCCCACGTTA CGTAACTTCC CATTTTAAGA 450
AAACTACAAT TCCCAACACA TACAAGTTAC TCCGCCCTAA AACCTACGTC 500
ACCCGCCCCG TTCCCACGCC CCGCGCCACG TCACAAACTC CACCCCCTCA 550
TTATCATATT GGCTTCAATC CAAAATAAGG TATATTATTG ATGATGCTAG 600
CATCATCAAT AATATACCTT ATTTTGGATT GAAGCCAATA TGATAATGAG 650
GGGGTGGAGT TTGTGACGTG GCGCGGGGCG TGGGAACGGG GCGGGTGACG 700
TAGTAGTGTG GCGGAAGTGT GATGTTGCAA GTGTGGCGGA ACACATGTAA 750
GCGACGGATG TGGCAAAAGT GACGTTTTTG GTGTGCGCCG GTGTACACAG 800
GAAGTGACAA TTTTCGCGCG GTTTTAGGCG GATGTTGTAG TAAATTTGGG 850
CGTAACCGAG TAAGATTTGG CCATTTTCGC GGGAAAACTG AATAAGAGGA 900
AGTGAAATCT GAATAATTTT GTGTTACTCA TAGCGCGTAA TATTTGTCTA 950
GGGAGATCAG CCTGCAGGTC GTTACATAAC TTACGGTAAA TGGCCCGCCT 1000
GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT 1050
TCCCATAGTA ACGCCAATAG GGACTTTCCA TTGACGTCAA TGGGTGGAGT 1100
ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA 1150
AGTACGCCCC CTATTGACGT CAATGACGGT AAATGGCCCG CCTGGCATTA 1200
TGCCCAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG 1250
TATTAGTCAT CGCTATTACC ATGGTGATGC GGTTTTGGCA GTACATCAAT 1300

FIGURE 5B

GGGCGTGGAT	AGCGGTTTGA	CTCACGGGGA	TTTCCAAGTC	TCCACCCCAT	1350
TGACGTCAAT	GGGAGTTTGT	TTTGGCACCA	AAATCAACGG	GACTTTCCAA	1400
AATGTCGTAA	CAACTCCGCC	CCATTGACGC	AAATGGGCGG	TAGGCGTGTA	1450
CGGTGGGAGG	TCTATATAAG	CAGAGCTCGT	TTAGTGAACC	GTCAGATCGC	1500
CTGGAGACGC	CATCCACGCT	GTTTTGACCT	CCATAGAAGA	CACCGGGACC	1550
GATCCAGCCT	CCGGACTCTA	GAGGATCCGG	TACTCGAGGA	ACTGAAAAAC	1600
CAGAAAGTTA	ACTGGTAAGT	TTAGTCTTTT	TGTCTTTTAT	TTCAGGTCCC	1650
GGATCCGGTG	GTGGTGCAAA	TCAAAGAACT	GCTCCTCAGT	GGATGTTGCC	1700
TTTACTTCTA	GGCCTGTACG	GAAGTGTTAC	TTCTGCTCTA	AAAGCTGCGG	1750
AATTGTACCC	GCGGCCGCAA	TTCCCGGGGA	TCGAAAGAGC	CTGCTAAAGC	1800
AAAAAAGAAG	TCACCATGTC	GTTTACTTTG	ACCAACAAGA	ACGTGATTTT	1850
CGTTGCCGGT	CTGGGAGGCA	TTGGTCTGGA	CACCAGCAAG	GAGCTGCTCA	1900
AGCGCGATCC	CGTCGTTTTA	CAACGTCGTG	ACTGGGAAAA	CCCTGGCGTT	1950
ACCCAACTTA	ATCGCCTTGC	AGCACATCCC	CCTTTCGCCA	GCTGGCGTAA	2000
TAGCGAAGAG	GCCCGCACCG	ATCGCCCTTC	CCAACAGTTG	CGCAGCCTGA	2050
ATGGCGAATG	GCGCTTTGCC	TGGTTTCCGG	CACCAGAAGC	GGTGCCGGAA	2100
AGCTGGCTGG	AGTGCGATCT	TCCTGAGGCC	GATACTGTCG	TCGTCCCCTC	2150
AAACTGGCAG	ATGCACGGTT	ACGATGCGCC	CATCTACACC	AACGTAACCT	2200
ATCCCATTAC	GGTCAATCCG	CCGTTTGTTC	CCACGGAGAA	TCCGACGGGT	2250
TGTTACTCGC	TCACATTTAA	TGTTGATGAA	AGCTGGCTAC	AGGAAGGCCA	2300
GACGCGAATT	ATTTTTGATG	GCGTTAACTC	GGCGTTTCAT	CTCTGGTGCA	2350
ACGGGCGCTG	GGTCGGTTAC	GGCCAGGACA	GTCGTTTGCC	GTCTGAATTT	2400
GACCTGAGCG	CATTTTTACG	CGCCGGAGAA	AACCGCCTCG	CGGTGATGGT	2450
GCTGCGTTGG	AGTGACGGCA	GTTATCTGGA	AGATCAGGAT	ATGTGGCGGA	2500
TGAGCGGCAT	TTTCCGTGAC	GTCTCGTTGC	TGCATAAACC	GACTACACAA	2550
ATCAGCGATT	TCCATGTTGC	CACTCGCTTT	AATGATGATT	TCAGCCGCGC	2600

FIGURE 5C

TGTACTGGAG	GCTGAAGTTC	AGATGTGCGG	CGAGTTGCGT	GACTACCTAC	2650
GGGTAACAGT	TTCTTTATGG	CAGGGTGAAA	CGCAGGTCGC	CAGCGGCACC	2700
GCGCCTTTCG	GCGGTGAAAT	TATCGATGAG	CGTGGTGGTT	ATGCCGATCG	2750
CGTCACACTA	CGTCTGAACG	TCGAAAACCC	GAAACTGTGG	AGCGCCGAAA	2800
TCCCGAATCT	CTATCGTGCG	GTGGTTGAAC	TGCACACCGC	CGACGGCACG	2850
CTGATTGAAG	CAGAAGCCTG	CGATGTCGGT	TTCCGCGAGG	TGCGGATTGA	2900
AAATGGTCTG	CTGCTGCTGA	ACGGCAAGCC	GTTGCTGATT	CGAGGCGTTA	2950
ACCGTCACGA	GCATCATCCT	CTGCATGGTC	AGGTCATGGA	TGAGCAGACC	3000
ATGGTGCAGG	ATATCCTGCT	GATGAAGCAG	AACAACTTTA	ACGCCGTGCG	3050
CTGTTCGCAT	TATCCGAACC	ATCCGCTGTG	GTACACGCTG	TGCGACCGCT	3100
ACGGCCTGTA	TGTGGTGGAT	GAAGCCAATA	TTGAAACCCA	CGGCATGGTG	3150
CCAATGAATC	GTCTGACCGA	TGATCCGCGC	TGGCTACCGG	CGATGAGCGA	3200
ACGCGTAACG	CGAATGGTGC	AGCGCGATCG	TAATCACCCG	AGTGTGATCA	3250
TCTGCTCGCT	GGGGAATGAA	TCAGGCCACG	GCGCTAATCA	CGACGCGCTG	3300
TATCGCTGGA	TCAAATCTGT	CGATCCTTCC	CGCCCGGTGC	AGTATGAAGG	3350
CGGCGGAGCC	GACACCACGG	CCACCGATAT	TATTTGCCCG	ATGTACGCGC	3400
GCGTGGATGA	AGACCAGCCC	TTCCCGGCTG	TGCCGAAATG	GTCCATCAAA	3450
AAATGGCTTT	CGCTACCTGG	AGAGACGCGC	CCGCTGATCC	TTTGCGAATA	3500
CGCCCACGCG	ATGGGTAACA	GTCTTGGCGG	TTTCGCTAAA	TACTGGCAGG	3550
CGTTTCGTCA	GTATCCCCGT	TTACAGGGCG	GCTTCGTCTG	GGACTGGGTG	3600
GATCAGTCGC	TGATTAAATA	TGATGAAAAC	GGCAACCCGT	GGTCGGCTTA	3650
CGGCGGTGAT	TTTGGCGATA	CGCCGAACGA	TCGCCAGTTC	TGTATGAACG	3700
GTCTGGTCTT	TGCCGACCGC	ACGCCGCATC	CAGCGCTGAC	GGAAGCAAAA	3750
CACCAGCAGC	AGTTTTTCCA	GTTCCGTTTA	TCCGGGCAAA	CCATCGAAGT	3800
GACCAGCGAA	TACCTGTTCC	GTCATAGCGA	TAACGAGCTC	CTGCACTGGA	3850
TGGTGGCGCT	GGATGGTAAG	CCGCTGGCAA	GCGGTGAAGT	GCCTCTGGAT	3900

FIGURE 5D

GTCGCTCCAC AAGGTAAACA GTTGATTGAA CTGCCTGAAC TACCGCAGCC 3950

GGAGAGCGCC GGGCAACTCT GGCTCACAGT ACGCGTAGTG CAACCGAACG 4000

CGACCGCATG GTCAGAAGCC GGGCACATCA GCGCCTGGCA GCAGTGGCGT 4050

CTGGCGGAAA ACCTCAGTGT GACGCTCCCC GCCGCGTCCC ACGCCATCCC 4100

GCATCTGACC ACCAGCGAAA TGGATTTTTG CATCGAGCTG GGTAATAAGC 4150

GTTGGCAATT TAACCGCCAG TCAGGCTTTC TTTCACAGAT GTGGATTGGC 4200

GATAAAAAAC AACTGCTGAC GCCGCTGCGC GATCAGTTCA CCCGTGCACC 4250

GCTGGATAAC GACATTGGCG TAAGTGAAGC GACCCGCATT GACCCTAACG 4300

CCTGGGTCGA ACGCTGGAAG GCGGCGGGCC ATTACCAGGC CGAAGCAGCG 4350

TTGTTGCAGT GCACGGCAGA TACACTTGCT GATGCGGTGC TGATTACGAC 4400

CGCTCACGCG TGGCAGCATC AGGGGAAAAC CTTATTTATC AGCCGGAAAA 4450

CCTACCGGAT TGATGGTAGT GGTCAAATGG CGATTACCGT TGATGTTGAA 4500

GTGGCGAGCG ATACACCGCA TCCGGCGCGG ATTGGCCTGA ACTGCCAGCT 4550

GGCGCAGGTA GCAGAGCGGG TAAACTGGCT CGGATTAGGG CCGCAAGAAA 4600

ACTATCCCGA CCGCCTTACT GCCGCCTGTT TTGACCGCTG GGATCTGCCA 4650

TTGTCAGACA TGTATACCCC GTACGTCTTC CCGAGCGAAA ACGGTCTGCG 4700

CTGCGGGACG CGCGAATTGA ATTATGGCCC ACACCAGTGG CGCGGCGACT 4750

TCCAGTTCAA CATCAGCCGC TACAGTCAAC AGCAACTGAT GGAAACCAGC 4800

CATCGCCATC TGCTGCACGC GGAAGAAGGC ACATGGCTGA ATATCGACGG 4850

TTTCCATATG GGGATTGGTG GCGACGACTC CTGGAGCCCG TCAGTATCGG 4900

CGGAATTACA GCTGAGCGCC GGTCGCTACC ATTACCAGTT GGTCTGGTGT 4950

CAAAAATAAT AATAACCGGG CAGGCCATGT CTGCCCGTAT TTCGCGTAAG 5000

GAAATCCATT ATGTACTATT TAAAAAACAC AAACTTTTGG ATGTTCGGTT 5050

TATTCTTTTT CTTTTACTTT TTTATCATGG GAGCCTACTT CCCGTTTTTC 5100

CCGATTTGGC TACATGACAT CAACCATATC AGCAAAAGTG ATACGGGTAT 5150

TATTTTTGCC GCTATTTCTC TGTTCTCGCT ATTATTCCAA CCGCTGTTTG 5200

GTCTGCTTTC TGACAAACTC GGCCTCGACT CTAGGCGGCC GCGGGGATCC 5250

FIGURE 5E

AGACATGATA AGATACATTG ATGAGTTTGG ACAAACCACA ACTAGAATGC 5300
AGTGAAAAAA ATGCTTTATT TGTGAAATTT GTGATGCTAT TGCTTTATTT 5350
GTAACCATTA TAAGCTGCAA TAAACAAGTT AACAACAACA ATTGCATTCA 5400
TTTTATGTTT CAGGTTCAGG GGGAGGTGTG GGAGGTTTTT TCGGATCCTC 5450
TAGAGTCGAC GACGCGAGGC TGGATGGCCT TCCCCATTAT GATTCTTCTC 5500
GCTTCCGGCG GCATCGGGAT GCCCGCGTTG CAGGCCATGC TGTCCAGGCA 5550
GGTAGATGAC GACCATCAGG GACAGCTTCA AGGATCGCTC GCGGCTCTTA 5600
CCAGCCTAAC TTCGATCACT GGACCGCTGA TCGTCACGGC GATTTATGCC 5650
GCCTCGGCGA GCACATGGAA CGGGTTGGCA TGGATTGTAG GCGCCGCCCT 5700
ATACCTTGTC TGCCTCCCCG CGTTGCGTCG CGGTGCATGG AGCCGGGCCA 5750
CCTCGACCTG AATGGAAGCC GGCGGCACCT CGCTAACGGA TTCACCACTC 5800
CAAGAATTGG AGCCAATCAA TTCTTGCGGA GAACTGTGAA TGCGCAAACC 5850
AACCCTTGGC AGAACATATC CATCGCGTCC GCCATCTCCA GCAGCCGCAC 5900
GCGGCGCATC TCGGGCAGCG TTGGGTCCTG GCCACGGGTG CGCATGATCG 5950
TGCTCCTGTC GTTGAGGACC CGGCTAGGCT GGCGGGGTTG CCTTACTGGT 6000
TAGCAGAATG AATCACCGAT ACGCGAGCGA ACGTGAAGCG ACTGCTGCTG 6050
CAAAACGTCT GCGACCTGAG CAACAACATG AATGGTCTTC GGTTTCCGTG 6100
TTTCGTAAAG TCTGGAAACG CGGAAGTCAG CGCCCTGCAC CATTATGTTC 6150
CGGATCTGCA TCGCAGGATG CTGCTGGCTA CCCTGTGGAA CACCTACATC 6200
TGTATTAACG AAGCCTTTCT CAATGCTCAC GCTGTAGGTA TCTCAGTTCG 6250
GTGTAGGTCG TTCGCTCCAA GCTGGGCTGT GTGCACGAAC CCCCCGTTCA 6300
GCCCGACCGC TGCGCCTTAT CCGGTAACTA TCGTCTTGAG TCCAACCCGG 6350
TAAGACACGA CTTATCGCCA CTGGCAGCAG CCACTGGTAA CAGGATTAGC 6400
AGAGCGAGGT ATGTAGGCGG TGCTACAGAG TTCTTGAAGT GGTGGCCTAA 6450
CTACGGCTAC ACTAGAAGGA CAGTATTTGG TATCTGCGCT CTGCTGAAGC 6500
CAGTTACCTT CGGAAAAAGA GTTGGTAGCT CTTGATCCGG CAAACAAACC 6550

FIGURE 5F

ACCGCTGGTA GCGGTGGTTT TTTTGTTTGC AAGCAGCAGA TTACGCGCAG 6600
AAAAAAAGGA TCTCAAGAAG ATCCTTTGAT CTTTTCTACG GGGTCTGACG 6650
CTCAGTGGAA CGAAAACTCA CGTTAAGGGA TTTTGGTCAT GAGATTATCA 6700
AAAAGGATCT TCACCTAGAT CCTTTTAAAT TAAAAATGAA GTTTTAAATC 6750
AATCTAAAGT ATATATGAGT AAACTTGGTC TGACAGTTAC CAATGCTTAA 6800
TCAGTGAGGC ACCTATCTCA GCGATCTGTC TATTTCGTTC ATCCATAGTT 6850
GCCTGACTCC CCGTCGTGTA GATAACTACG ATACGGGAGG GCTTACCATC 6900
TGGCCCCAGT GCTGCAATGA TACCGCGAGA CCCACGCTCA CCGGCTCCAG 6950
ATTTATCAGC AATAAACCAG CCAGCCGGAA GGGCCGAGCG CAGAAGTGGT 7000
CCTGCAACTT TATCCGCCTC CATCCAGTCT ATTAATTGTT GCCGGGAAGC 7050
TAGAGTAAGT AGTTCGCCAG TTAATAGTTT GCGCAACGTT GTTGCCATTG 7100
CTGCAGGCAT CGTGGTGTCA CGCTCGTCGT TTGGTATGGC TTCATTCAGC 7150
TCCGGTTCCC AACGATCAAG GCGAGTTACA TCATCCCCCA TGTTGTGCAA 7200
AAAAGCGGTT AGCTCCTTCG GTCCTCCGAT CGTTGTCAGA AGTAAGTTGG 7250
CCGCAGTGTT ATCACTCATG GTTATGCCAG CACTGCATAA TTCTCTTACT 7300
GTCATGCCAT CCGTAAGATG CTTTTCTGTG ACTGGTGAGT ACTCAACCAA 7350
GTCATTCTGA GAATAGTGTA TGCGGCGACC GAGTTGCTCT TGCCCGGCGT 7400
CAACACGGGA TAATACCGCG CCACATAGCA CAACTTTAAA AGTGCTCATC 7450
ATTGGAAAAC GTTCTTCGGG GCGAAAACTC TCAAGGATCT TACCGCTGTT 7500
GAGATCCAGT TCGATGTAAC CCACTCGTGC ACCCAACTGA TCTTCAGCAT 7550
CTTTTACTTT CACCAGCGTT TCTGGGTGAG CAAAAACAGG AAGGCAAAAT 7600
GCCGCAAAAA AGGGAATAAG GGCGACACGG AAATGTTGAA TACTCATACT 7650
CTTCCTTTTT CAATATTATT GAAGCATTTA TCAGGGTTAT TGTCTCATGA 7700
GCGGATACAT ATTTGAATGT ATTTAGAAAA ATAAACAAAT AGGGGTTCCG 7750
CGCACATTTC CCCGAAAAGT GCCACCTGAC GTCTAAGAAA CCATTATTAT 7800
CATGACATTA ACCTATAAAA ATAGGCGTAT CACGAGGCCC TTTCGTCTTC 7850
AA 7852

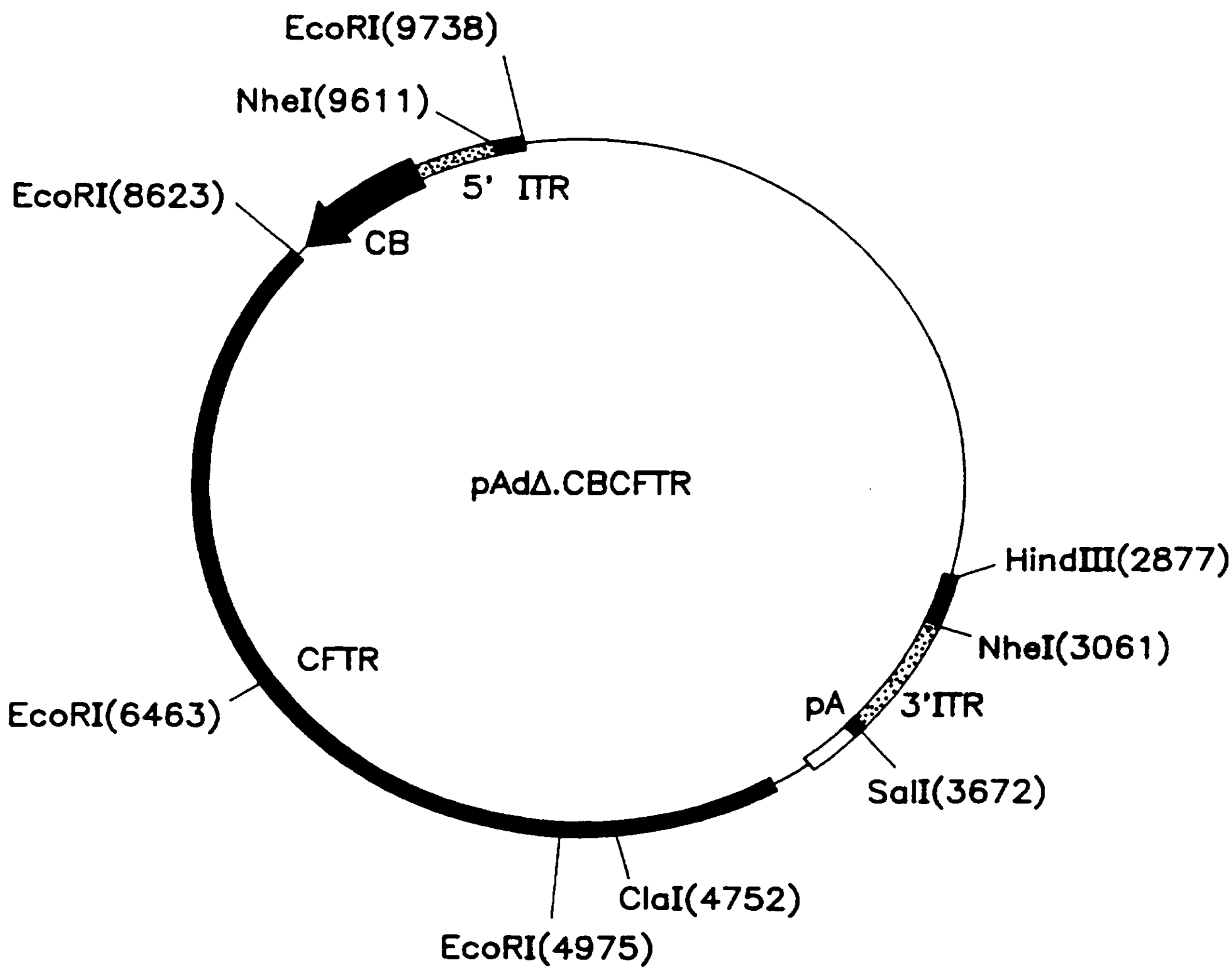
EcoRI(9738)
NheI(9611)
EcoRI(8623)
5' ITR
CB
pAdΔ.CBCFTR
HindIII(2877)
NheI(3061)
CFTR
pA
3'ITR
EcoRI(6463)
SalI(3672)
ClaI(4752)
EcoRI(4975)

FIG. 6

FIGURE 7A

```
TCTTCCGCTT CCTCGCTCAC TGACTCGCTG CGCTCGGTCG TTCGGCTGCG    50
GCGAGCGGTA TCAGCTCACT CAAAGGCGGT AATACGGTTA TCCACAGAAT   100
CAGGGGATAA CGCAGGAAAG AACATGTGAG CAAAAGGCCA GCAAAAGGCC   150
AGGAACCGTA AAAAGGCCGC GTTGCTGGCG TTTTTCCATA GGCTCCGCCC   200
CCCTGACGAG CATCACAAAA ATCGACGCTC AAGTCAGAGG TGGCGAAACC   250
CGACAGGACT ATAAAGATAC CAGGCGTTTC CCCCTGGAAG CTCCCTCGTG   300
CGCTCTCCTG TTCCGACCCT GCCGCTTACC GGATACCTGT CCGCCTTTCT   350
CCCTTCGGGA AGCGTGGCGC TTTCTCATAG CTCACGCTGT AGGTATCTCA   400
GTTCGGTGTA GGTCGTTCGC TCCAAGCTGG GCTGTGTGCA CGAACCCCCC   450
GTTCAGCCCG ACCGCTGCGC CTTATCCGGT AACTATCGTC TTGAGTCCAA   500
CCCGGTAAGA CACGACTTAT CGCCACTGGC AGCAGCCACT GGTAACAGGA   550
TTAGCAGAGC GAGGTATGTA GGCGGTGCTA CAGAGTTCTT GAAGTGGTGG   600
CCTAACTACG GCTACACTAG AAGAACAGTA TTTGGTATCT GCGCTCTGCT   650
GAAGCCAGTT ACCTTCGGAA AAAGAGTTGG TAGCTCTTGA TCCGGCAAAC   700
AAACCACCGC TGGTAGCGGT GGTTTTTTTG TTTGCAAGCA GCAGATTACG   750
CGCAGAAAAA AAGGATCTCA AGAAGATCCT TTGATCTTTT CTACGGGGTC   800
TGACGCTCAG TGGAACGAAA ACTCACGTTA AGGGATTTTG GTCATGAGAT   850
TATCAAAAAG GATCTTCACC TAGATCCTTT TAAATTAAAA ATGAAGTTTT   900
AAATCAATCT AAAGTATATA TGAGTAAACT TGGTCTGACA GTTACCAATG   950
CTTAATCAGT GAGGCACCTA TCTCAGCGAT CTGTCTATTT CGTTCATCCA  1000
TAGTTGCCTG ACTCCCCGTC GTGTAGATAA CTACGATACG GGAGGGCTTA  1050
CCATCTGGCC CCAGTGCTGC AATGATACCG CCAGACCCAC GCTCACCGGC  1100
TCCAGATTTA TCAGCAATAA ACCAGCCAGC CGGAAGGGCC GAGCGCAGAA  1150
GTGGTCCTGC AACTTTATCC GCCTCCATCC AGTCTATTAA TTGTTGCCGG  1200
GAAGCTAGAG TAAGTAGTTC GCCAGTTAAT AGTTTGCGCA ACGTTGTTGC  1250
CATTGCTACA GGCATCGTGG TGTCACGCTC GTCGTTTGGT ATGGCTTCAT  1300
```

FIGURE 7B

TCAGCTCCGC	TTCCCAACGA	TCAAGGCGAG	TTACATGATC	CCCCATGTTG	1350
TGCAAAAAAG	CGGTTAGCTC	CTTCGGTCCT	CCGATCGTTG	TCAGAAGTAA	1400
GTTGGCCGCA	GTGTTATCAC	TCATGGTTAT	GGCAGCACTG	CATAATTCTC	1450
TTACTGTCAT	GCCATCCGTA	AGATGCTTTT	CTGTGACTGG	TGAGTACTCA	1500
ACCAAGTCAT	TCTGAGAATA	GTGTATGCGG	CGACCGAGTT	GCTCTTGCCC	1550
GGCGTCAATA	CGGGATAATA	CCGCGCCACA	TAGCAGAACT	TTAAAAGTGC	1600
TCATCATTGG	AAAACGTTCT	TCGGGGCGAA	AACTCTCAAG	GATCTTACCG	1650
CTGTTGAGAT	CCAGTTCGAT	GTAACCCACT	CGTGCACCCA	ACTGATCTTC	1700
AGCATCTTTT	ACTTTCACCA	GCGTTTCTGG	GTGAGCAAAA	ACAGGAAGGC	1750
AAAATGCCGC	AAAAAAGGGA	ATAAGGGCGA	CACGGAAATG	TTGAATACTC	1800
ATACTCTTCC	TTTTTCAATA	TTATTGAAGC	ATTTATCAGG	GTTATTGTCT	1850
CATGAGCGGA	TACATATTTG	AATGTATTTA	GAAAAATAAA	CAAATAGGGG	1900
TTCCGCGCAC	ATTTCCCCGA	AAAGTGCCAC	CTGACGTCTA	AGAAACCATT	1950
ATTATCATGA	CATTAACCTA	TAAAAATAGG	CGTATCACGA	GGCCCTTTCG	2000
TCTCGCGCGT	TTCGGTGATG	ACGGTGAAAA	CCTCTGACAC	ATGCAGCTCC	2050
CGGAGACGGT	CACAGCTTGT	CTGTAAGCGG	ATGCCGGGAG	CAGACAAGCC	2100
CGTCAGGGCG	CGTCAGCGGG	TGTTGGCGGG	TGTCGGGGCT	GGCTTAACTA	2150
TGCGGCATCA	GAGCAGATTG	TACTGAGAGT	GCACCATAAA	ATTGTAAACG	2200
TTAATATTTT	GTTAAAATTC	GCGTTAAATT	TTTGTTAAAT	CAGCTCATTT	2250
TTTAACCAAT	AGGCCGAAAT	CGGCAAAATC	CCTTATAAAT	CAAAAGAATA	2300
GCCCGAGATA	GGGTTGAGTG	TTGTTCCAGT	TTGGAACAAG	AGTCCACTAT	2350
TAAAGAACGT	GGACTCCAAC	GTCAAAGGGC	GAAAAACCGT	CTATCAGGGC	2400
GATGGCCCAC	TACGTGAACC	ATCACCCAAA	TCAAGTTTTT	TGGGGTCGAG	2450
GTGCCGTAAA	GCACTAAATC	GGAACCCTAA	AGGGAGCCCC	CGATTTAGAG	2500
CTTGACGGGG	AAAGCCGGCG	AACGTGGCGA	GAAAGGAAGG	GAAGAAAGCG	2550
AAAGGAGCGG	GCGCTAGGGC	GCTGGCAAGT	GTAGCGGTCA	CGCTGCGCGT	2600

FIGURE 7C

AACCACCACA	CCCGCCGCGC	TTAATGCGCC	GCTACAGGGC	GCGTACTATG	2650
GTTGCTTTGA	CGTATGCGGT	GTGAAATACC	GCACAGATGC	GTAAGGAGAA	2700
AATACCGCAT	CAGGCGCCAT	TCGCCATTCA	GGCTGCGCAA	CTGTTGGGAA	2750
GGGCGATCGG	TGCGGGCCTC	TTCGCTATTA	CGCCAGCTGG	CGAAAGGGGG	2800
ATGTGCTGCA	AGGCGATTAA	GTTGGGTAAC	GCCAGGGTTT	TCCCAGTCAC	2850
GACGTTGTAA	AACGACGGCC	AGTGCCAAGC	TTAAGGTGCA	CGGCCCACGT	2900
GGCCACTAGT	ACTTCTCGAG	CTCTGTACAT	GTCCGCGGTC	GCGACGTACG	2950
CGTATCGATG	GCGCCAGCTG	CAGGCGGCCG	CCATATGCAT	CCTAGGCCTA	3000
TTAATATTCC	GGAGTATACG	TAGCCGGCTA	ACGTTAACAA	CCGGTACCTC	3050
TAGAACTATA	GCTAGCCAAT	TCCATCATCA	ATAATATACC	TTATTTTGGA	3100
TTGAAGCCAA	TATGATAATG	AGGGGGTGGA	GTTTGTGACG	TGGCGCGGGG	3150
CGTGGGAACG	GGGCGGGTGA	CGTAGGTTTT	AGGGCGGAGT	AACTTGTATG	3200
TGTTGGGAAT	TGTAGTTTTC	TTAAAATGGG	AAGTTACGTA	ACGTGGGAAA	3250
ACGGAAGTGA	CGATTTGAGG	AAGTTGTGGG	TTTTTTGGCT	TTCGTTTCTC	3300
GGCGTAGGTT	CGCGTGCGGT	TTTCTGGGTG	TTTTTTGTGG	ACTTTAACCG	3350
TTACGTCATT	TTTTAGTCCT	ATATATACTC	GCTCTGCACT	TGGCCCTTTT	3400
TTACACTGTG	ACTGATTGAG	CTGGTGCCGT	GTCGAGTGGT	GTTTTTTTAA	3450
TAGGTTTTCT	TTTTTACTGG	TAAGGCTGAC	TGTTAGGCTG	CCGCTGTGAA	3500
GCGCTGTATG	TTGTTCTGGA	GCGGGAGGGT	GCTATTTTGC	CTAGGCAGGA	3550
GGGTTTTTCA	GGTGTTTATG	TGTTTTTCTC	TCCTATTAAT	TTTGTTATAC	3600
CTCCTATGGG	GGCTGTAATG	TTGTCTCTAC	GCCTGCGGGT	ATGTATTCCC	3650
CCCAAGCTTG	CATGCCTGCA	GGTCGACTCT	AGAGGATCCG	AAAAAACCTC	3700
CCACACCTCC	CCCTGAACCT	GAAACATAAA	ATGAATGCAA	TTGTTGTTGT	3750
TAACTTGTTT	ATTGCAGCTT	ATAATGGTTA	CAAATAAAGC	AATAGCATCA	3800
CAAATTTCAC	AAATAAAGCA	TTTTTTTCAC	TGCATTCTAG	TTGTGGTTTG	3850
TCCAAACTCA	TCAATGTATC	TTATCATGTC	TGGATCCCCC	TAGCTTGCCA	3900

FIGURE 7D

AACCTACAGG	TGGGGTCTTT	CATTCCCCCC	TTTTTCTGGA	GACTAAATAA	3950
AATCTTTTAT	TTTATCTATG	GCTCGTACTC	TATAGGCTTC	AGCTGGTGAT	4000
ATTGTTGAGT	CAAAACTAGA	GCCTGGACCA	CTGATATCCT	GTCTTTAACA	4050
AATTGGACTA	ATCGCGGGAT	CAGCCAATTC	CATGAGCAAA	TGTCCCATGT	4100
CAACATTTAT	GCTGCTCTCT	AAAGCCTTGT	ATCTTGCATC	TCTTCTTCTG	4150
TCTCCTCTTT	CAGAGCAGCA	ATCTGGGGCT	TAGACTTGCA	CTTGCTTGAG	4200
TTCCGGTGGG	GAAAGAGCTT	CACCCTGTCG	GAGGGGCTGA	TGGCTTGCCG	4250
GAAGAGGCTC	CTCTCGTTCA	GCAGTTTCTG	GATGGAATCG	TACTGCCGCA	4300
CTTTGTTCTC	TTCTATGACC	AAAAATTGTT	GGCATTCCAG	CATTGCTTCT	4350
ATCCTGTGTT	CACAGAGAAT	TACTGTGCAA	TCAGCAAATG	CTTGTTTTAG	4400
AGTTCTTCTA	ATTATTTGGT	ATGTTACTGG	ATCCAAATGA	GCACTGGGTT	4450
CATCAAGCAG	CAAGATCTTC	GCCTTACTGA	GAACAGATCT	AGCCAAGCAC	4500
ATCAACTGCT	TGTGGCCATG	GCTTAGGACA	CAGCCCCCAT	CCACAAGGAC	4550
AAAGTCAAGC	TTCCCAGGAA	ACTGTTCTAT	CACAGATCTG	AGCCCAACCT	4600
CATCTGCAAC	TTTCCATATT	TCTTGATCAC	TCCACTGTTC	ATAGGGATCC	4650
AAGTTTTTTC	TAAATGTTCC	AGAAAAAATA	AATACTTTCT	GTGGTATCAC	4700
TCCAAAGGCT	TTCCTCCACT	GTTGCAAAGT	TATTGAATCC	CAAGACACAC	4750
CATCGATCTG	GATTTCTCCT	TCAGTGTTCA	GTAGTCTCAA	AAAAGCTGAT	4800
AACAAAGTAC	TCTTCCCTGA	TCCAGTTCTT	CCCAAGAGGC	CCACCCTCTG	4850
GCCAGGACTT	ATTGAGAAGG	AAATGTTCTC	TAATATGGCA	TTTCCACCTT	4900
CTGTGTATTT	TGCTGTGAGA	TCTTTGACAG	TCATTTGGCC	CCCTGAGGGC	4950
CAGATGTCAT	CTTTCTTCAC	GTGTGAATTC	TCAATAATCA	TAACTTTCGA	5000
GAGTTGGCCA	TTCTTGTATG	GTTTGGTTGA	CTTGGTAGGT	TTACCTTCTG	5050
TTGGCATGTC	AATGAACTTA	AAGACTCGGC	TCACAGATCG	CATCAAGCTA	5100
TCCACATCTA	TGCTGGAGTT	TACAGCCCAC	TGCAATGTAC	TCATGATATT	5150
CATGGCTAAA	GTCAGGATAA	TACCAACTCT	TCCTTCTCCT	TCTCCTGTTG	5200

FIGURE 7E

TTAAAATGGA AATGAAGGTA ACAGCAATGA AGAAGATGAC AAAAATCATT 5250

TCTATTCTCA TTTGGAACCA GCGCAGTGTT GACAGGTACA AGAACCAGTT 5300

GGCAGTATGT AAATTCAGAG CTTTGTGGAA CAGAGTTTCA AAGTAAGGCT 5350

GCCGTCCGAA GGCACGAAGT GTCCATAGTC CTTTTAAGCT TGTAACAAGA 5400

TGAGTGAAAA TTGGACTCCT GCCTTCAGAT TCCAGTTGTT TGAGTTGCTG 5450

TGAGGTTTGG AGGAAATATG CTCTCAACAT AATAAAAGCC ACTATCACTG 5500

GCACTGTTGC AACAAAGATG TAGGGTTGTA AAACTGCGAC AACTGCTATA 5550

GCTCCAATCA CAATTAATAA CAACTGGATG AAGTCAAATA TGGTAAGAGG 5600

CAGAAGGTCA TCCAAAATTG CTATATCTTT GGAGAATCTA TTAAGAATCC 5650

CACCTGCTTT CAACGTGTTG AGGGTTGACA TAGGTGCTTG AAGAACAGAA 5700

TGTAACATTT TGTGGTGTAA AATTTTCGAC ACTGTGATTA GAGTATGCAC 5750

CAGTGGTAGA CCTCTGAAGA ATCCCATAGC AAGCAAAGTG TCGGCTACTC 5800

CCACGTAAAT GTAAAACACA TAATACGAAC TGGTGCTGGT GATAATCACT 5850

GCATAGCTGT TATTTCTACT ATGAGTACTA TTCCCTTTGT CTTGAAGAGG 5900

AGTGTTTCCA AGGAGCCACA GCACAACCAA AGAAGCAGCC ACCTCTGCCA 5950

GAAAAATTAC TAAGCACCAA ATTAGCACAA AAATTAAGCT CTTGTGGACA 6000

GTAATATATC GAAGGTATGT GTTCCATGTA GTCACTGCTG GTATGCTCTC 6050

CATATCATCA AAAAAGCACT CCTTTAAGTC TTCTTCGTTA ATTTCTTCAC 6100

TTATTTCCAA GCCAGTTTCT TGAGATAACC TTCTTGAATA TATATCCAGT 6150

TCAGTCAAGT TTGCCTGAGG GGCCAGTGAC ACTTTTCGTG TGGATGCTGT 6200

TGTCTTTCGG TGAATGTTCT GACCTTGGTT AACTGAGTGT GTCATCAGGT 6250

TCAGGACAGA CTGCCTCCTT CGTGCCTGAA GCGTGGGGCC AGTGCTGATC 6300

ACGCTGATGC GAGGCAGTAT CGCCTCTCCC TGCTCAGAAT CTGGTACTAA 6350

GGACAGCCTT CTCTCTAAAG GCTCATCAGA ATCCTCTTCG ATGCCATTCA 6400

TTTGTAAGGG AGTCTTTTGC ACAATGGAAA ATTTTCGTAT AGAGTTGATT 6450

GGATTGAGAA TAGAATTCTT CCTTTTTTCC CCAAACTCTC CAGTCTGTTT 6500

FIGURE 7F

AAAAGATTGT TTTTTTGTTT CTGTCCAGGA GACAGGAGCA TCTCCTTCTA 6550
ATGAGAAACG GTGTAAGGTC TCAGTTAGGA TTGAATTTCT TCTTTCTGCA 6600
CTAAATTGGT CGAAAGAATC ACATCCCATG AGTTTTGAGC TAAAGTCTGG 6650
CTGTAGATTT TGGAGTTCTG AAAATGTCCC ATAAAAATAG CTGCTACCTT 6700
CATGCAAAAT TAATATTTTG TCAGCTTTCT TTAAATGTTC CATTTTAGAA 6750
GTGACCAAAA TCCTAGTTTT GTTAGCCATC AGTTTACAGA CACAGCTTTC 6800
AAATATTTCT TTTTCTGTTA AAACATCTAG GTATCCAAAA GGAGAGTCTA 6850
ATAAATACAA ATCAGCATCT TTGTATACTG CTCTTGCTAA AGAAATTCTT 6900
GCTCGTTGAC CTCCACTCAG TGTGATTCCA CCTTCTCCAA GAACTATATT 6950
GTCTTTCTCT GCAAACTTGG AGATGTCCTC TTCTAGTTGG CATGCTTTGA 7000
TGACGCTTCT GTATCTATAT TCATCATAGG AAACACCAAA GATGATATTT 7050
TCTTTAATGG TGCCAGGCAT AATCCAGGAA AACTGAGAAC AGAATGAAAT 7100
TCTTCCACTG TGCTTAATTT TACCCTCTGA AGGCTCCAGT TCTCCCATAA 7150
TCATCATTAG AAGTGAAGTC TTGCCTGCTC CAGTGGATCC AGCAACCGCC 7200
AACAACTGTC CTCTTTCTAT CTTGAAATTA ATATCTTTCA GGACAGGAGT 7250
ACCAAGAAGT GAGAAATTAC TGAAGAAGAG GCTGTCATCA CCATTAGAAG 7300
TTTTTCTATT GTTATTGTTT TGTTTTGCTT TCTCAAATAA TTCCCCAAAT 7350
CCCTCCTCCC AGAAGGCTGT TACATTCTCC ATCACTACTT CTGTAGTCGT 7400
TAAGTTATAT TCCAATGTCT TATATTCTTG CTTTTGTAAG AAATCCTGTA 7450
TTTTGTTTAT TGCTCCAAGA GAGTCATACC ATGTTTGTAC AGCCCAGGGA 7500
AATTGCCGAG TGACCGCCAT GCGCAGAACA ATGCAGAATG AGATGGTGGT 7550
GAATATTTTC CGGAGGATGA TTCCTTTGAT TAGTGCATAG GGAAGCACAG 7600
ATAAAAACAC CACAAAGAAC CCTGAGAAGA AGAAGGCTGA GCTATTGAAG 7650
TATCTCACAT AGGCTGCCTT CCGAGTCAGT TTCAGTTCTG TTTGTCTTAA 7700
GTTTTCAATC ATTTTTTCCA TTGCTTCTTC CCAGCAGTAT GCCTTAACAG 7750
ATTGGATGTT CTCGATCATT TCTGAGGTAA TCACAAGTCT TTCACTGATC 7800

FIGURE 7G

TTCCCAGCTC	TCTGATCTCT	GTACTTCATC	ATCATTCTCC	CTAGCCCAGC	7850
CTGAAAAAGG	GCAAGGACTA	TCAGGAAACC	AAGTCCACAG	AAGGCAGACG	7900
CCTGTAACAA	CTCCCAGATT	AGCCCCATGA	GGAGTGCCAC	TTGCAAAGGA	7950
GCGATCCACA	CGAAATGTGC	CAATGCAAGT	CCTTCATCAA	ATTTGTTCAG	8000
GTTGTTGGAA	AGGAGACTAA	CAAGTTGTCC	AATACTTATT	TTATCTAGAA	8050
CACGGCTTGA	CAGCTTTAAA	GTCTTCTTAT	AAATCAAACT	AAACATAGCT	8100
ATTCTCATCT	GCATTCCAAT	GTGATGAAGG	CCAAAAATGG	CTGGGTGTAG	8150
GAGCAGTGTC	CTCACAATAA	AGAGAAGGCA	TAAGCCTATG	CCTAGATAAA	8200
TCGCGATAGA	GCGTTCCTCC	TTGTTATCCG	GGTCATAGGA	AGCTATGATT	8250
CTTCCCAGTA	AGAGAGGCTG	TACTGCTTTG	GTGACTTCCC	CTAAATATAA	8300
AAAGATTCCA	TAGAACATAA	ATCTCCAGAA	AAAACATCGC	CGAAGGGCAT	8350
TAATGAGTTT	AGGATTTTTC	TTTGAAGCCA	GCTCTCTATC	CCATTCTCTT	8400
TCCAATTTTT	CAGATAGATT	GTCAGCAGAA	TCAACAGAAG	GGATTTGGTA	8450
TATGTCTGAC	AATTCCAGGC	GCTGTCTGTA	TCCTTTCCTC	AAAATTGGTC	8500
TGGTCCAGCT	GAAAAAAAGT	TTGGAGACAA	CGCTGGCCTT	TTCCAGAGGC	8550
GACCTCTGCA	TGGTCTCTCG	GGCGCTGGGG	TCCCTGCTAG	GGCCGTCTGG	8600
GCTCAAGCTC	CTAATGCCAA	AGGAATTCCT	GCAGCCCGGG	GGATCCACTA	8650
GTTCTAGAGC	GGCCGCCACC	GCGGTGGCTG	ATCCCGCTCC	CGCCCGCCGC	8700
GCGCTTCGCT	TTTTATAGGG	CCGCCGCCGC	CGCCGCCTCG	CCATAAAAGG	8750
AAACTTTCGG	AGCGCGCCGC	TCTGATTGGC	TGCCGCCGCA	CCTCTCCGCC	8800
TCGCCCCGCC	CCGCCCCTCG	CCCCGCCCCG	CCCCGCCTGG	CGCGCGCCCC	8850
CCCCCCCCCC	CCGCCCCCAT	CGCTGCACAA	AATAATTAAA	AAATAAATAA	8900
ATACAAAATT	GGGGGTGGGG	AGGGGGGGGA	GATGGGGAGA	GTGAAGCAGA	8950
ACGTGGCCTC	GAGTAGATGT	ACTGCCAAGT	AGGAAAGTCC	CATAAGGTCA	9000
TGTACTGGGC	ATAATGCCAG	GCGGGCCATT	TACCGTCATT	GACGTCAATA	9050
GGGGGCGTAC	TTGGCATATG	ATACACTTGA	TGTACTGCCA	AGTGGGCAGT	9100

FIGURE 7H

TTACCGTAAA TACTCCACCC ATTGACGTCA ATGGAAAGTC CCTATTGGCG 9150
TTACTATGGG AACATACGTC ATTATTGACG TCAATGGGCG GGGGTCGTTG 9200
GGCGGTCAGC CAGGCGGGCC ATTTACCGTA AGTTATGTAA CGACCTGCAG 9250
GCTGATCTCC CTAGACAAAT ATTACGCGCT ATGAGTAACA CAAAATTATT 9300
CAGATTTCAC TTCCTCTTAT TCAGTTTTCC CGCGAAAATG GCCAAATCTT 9350
ACTCGGTTAC GCCCAAATTT ACTACAACAT CCGCCTAAAA CCGCGCGAAA 9400
ATTGTCACTT CCTGTGTACA CCGGCGCACA CCAAAAACGT CACTTTTGCC 9450
ACATCCGTCG CTTACATGTG TTCCGCCACA CTTGCAACAT CACACTTCCG 9500
CCACACTACT ACGTCACCCG CCCCGTTCCC ACGCCCCGCG CCACGTCACA 9550
AACTCCACCC CCTCATTATC ATATTGGCTT CAATCCAAAA TAAGGTATAT 9600
TATTGATGAT GCTAGCATGC GCAAATTTAA AGCGCTGATA TCGATCGCGC 9650
GCAGATCTGT CATGATGATC ATTGCAATTG GATCCATATA TAGGGCCCGG 9700
GTTATAATTA CCTCAGGTCG ACGTCCCATG GCCATTCGAA TTCGTAATCA 9750
TGGTCATAGC TGTTTCCTGT GTGAAATTGT TATCCGCTCA CAATTCCACA 9800
CAACATACGA GCCGGAAGCA TAAAGTGTAA AGCCTGGGGT GCCTAATGAG 9850
TGAGCTAACT CACATTAATT GCGTTGCGCT CACTGCCCGC TTTCCAGTCG 9900
GGAAACCTGT CGTGCCAGCT GCATTAATGA ATCGGCCAAC GCGCGGGGAG 9950
AGGCGGTTTG CGTATTGGGC GC 9972

 SUBSTITUTE SHEET (RULE 26)

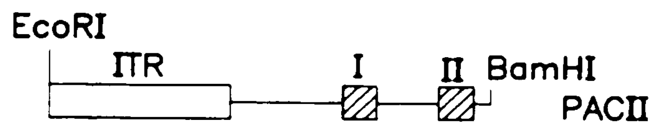

FIG. 8A

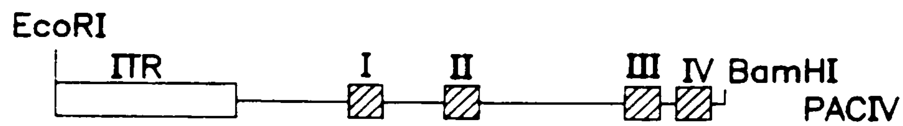

FIG. 8B

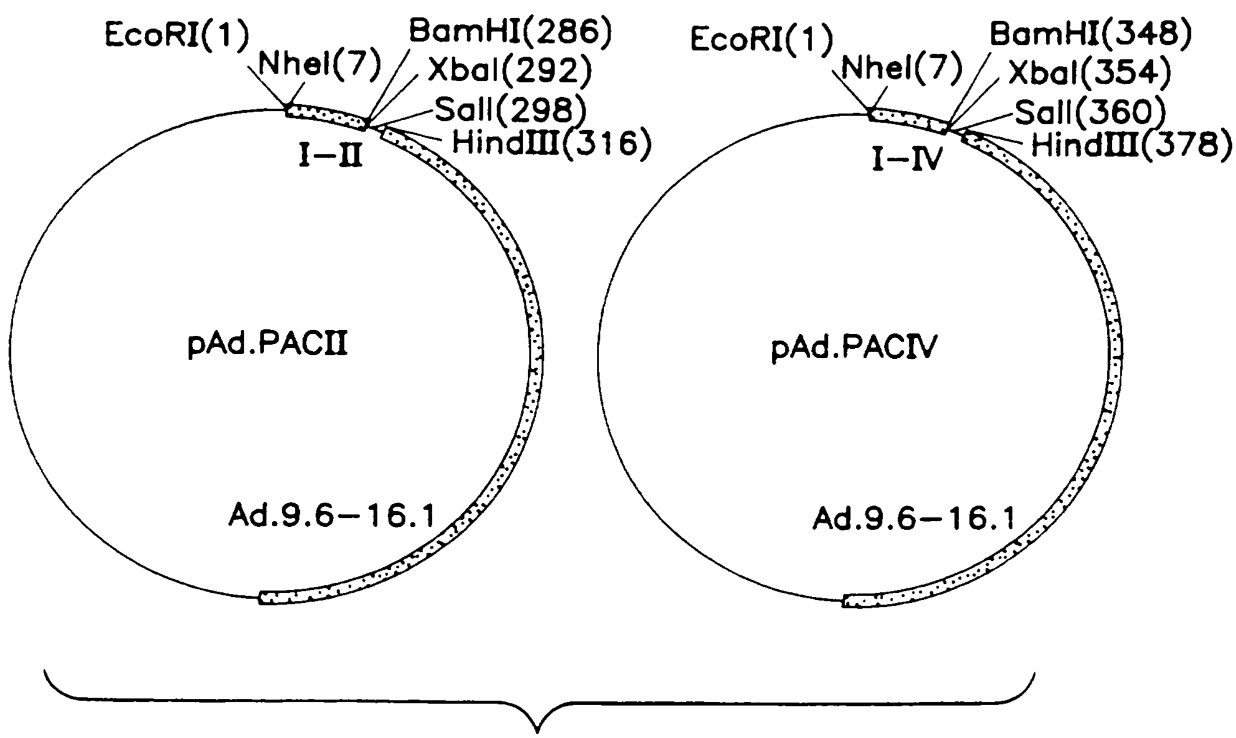

FIG. 8C

SUBSTITUTE SHEET (RULE 26)

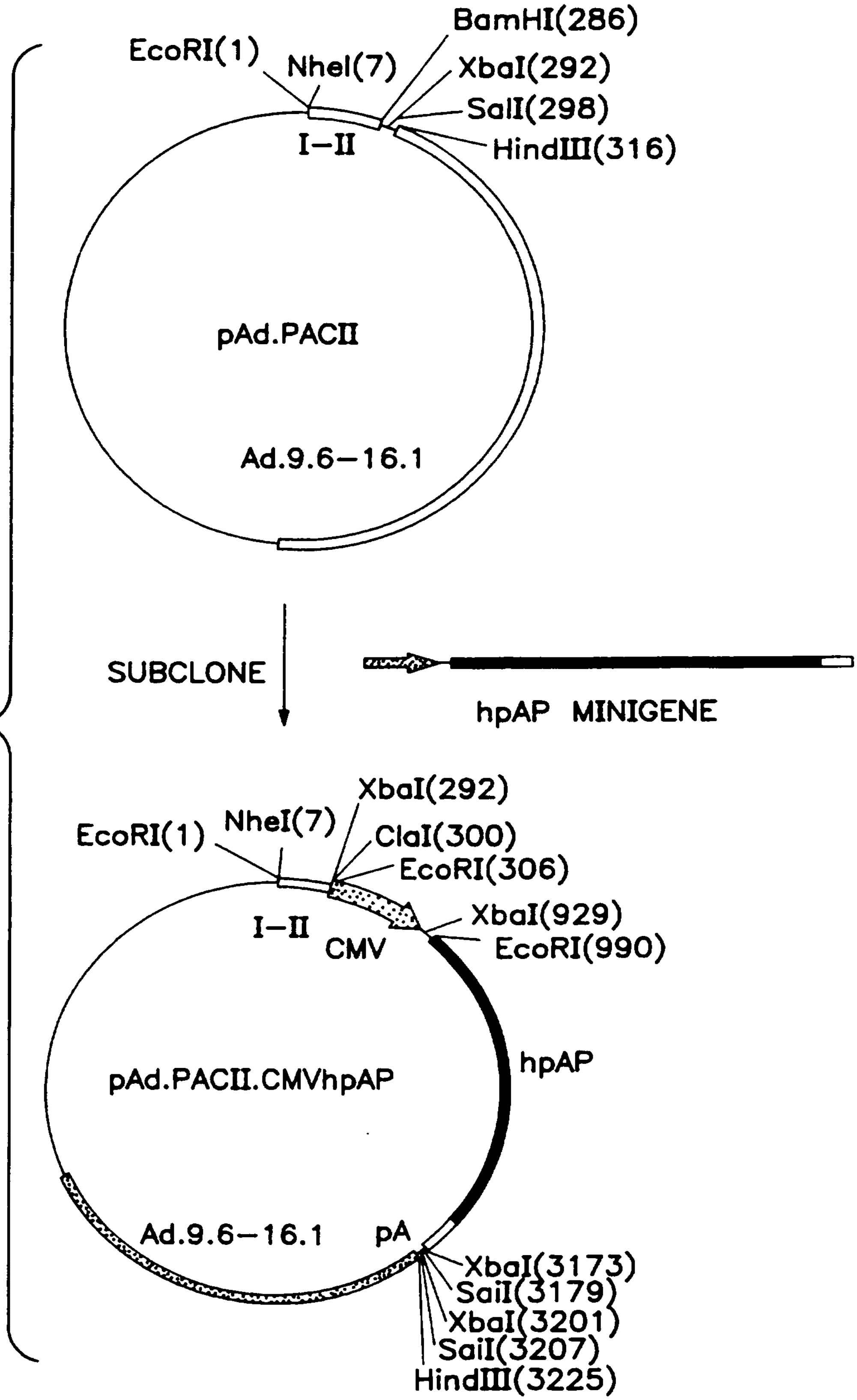
EcoRI(1)
NheI(7)
BamHI(286)
XbaI(292)
SalI(298)
HindIII(316)
I–II
pAd.PACII
Ad.9.6–16.1
SUBCLONE
hpAP MINIGENE
EcoRI(1)
NheI(7)
XbaI(292)
ClaI(300)
EcoRI(306)
XbaI(929)
EcoRI(990)
I–II
CMV
hpAP
pAd.PACII.CMVhpAP
Ad.9.6–16.1
pA
XbaI(3173)
SalI(3179)
XbaI(3201)
SalI(3207)
HindIII(3225)

FIG. 9A

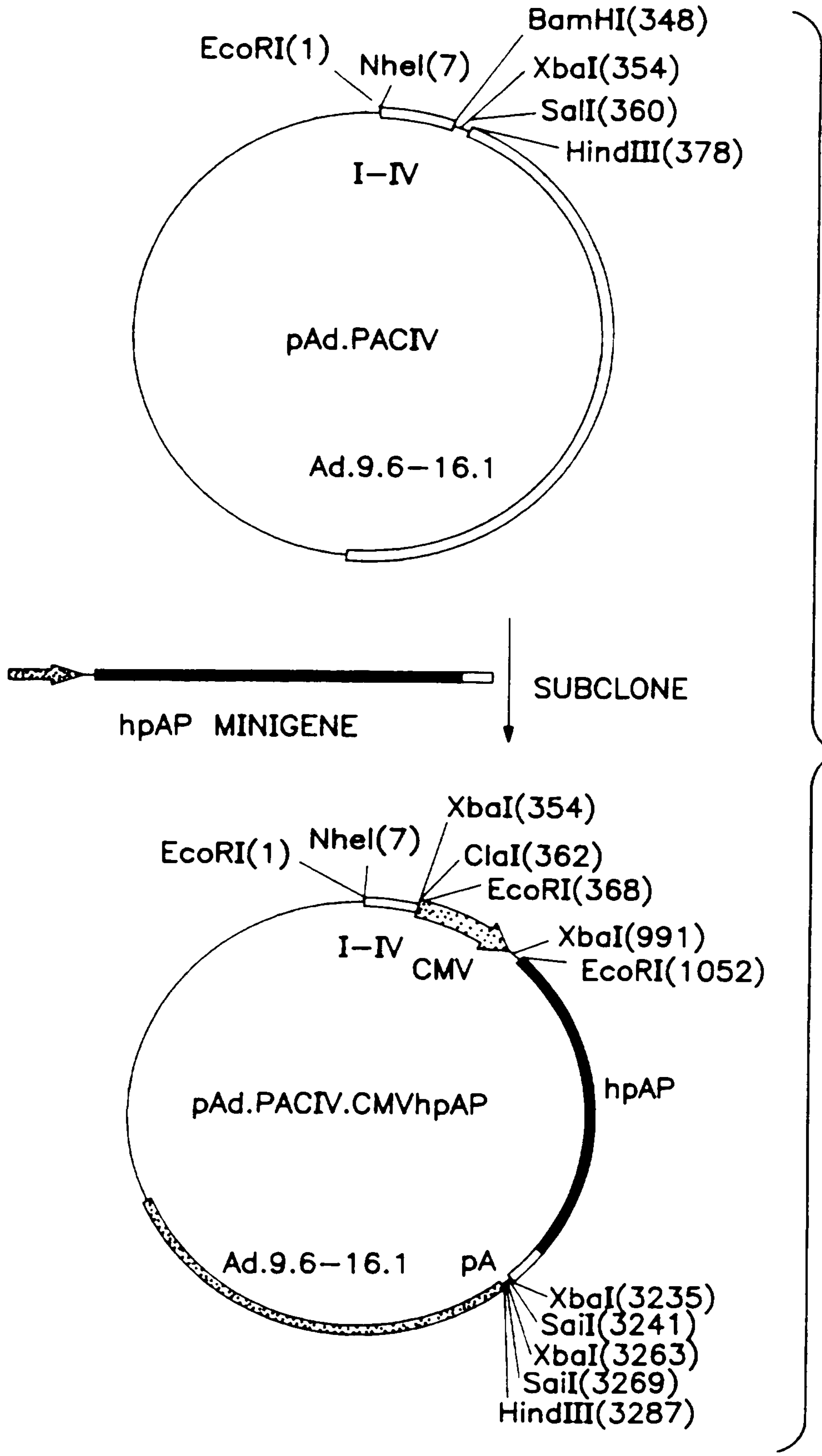
EcoRI(1)
NheI(7)
BamHI(348)
XbaI(354)
SalI(360)
HindIII(378)
I–IV
pAd.PACIV
Ad.9.6–16.1
SUBCLONE
hpAP MINIGENE
EcoRI(1)
NheI(7)
XbaI(354)
ClaI(362)
EcoRI(368)
XbaI(991)
EcoRI(1052)
I–IV
CMV
hpAP
pAd.PACIV.CMVhpAP
Ad.9.6–16.1
pA
XbaI(3235)
SalI(3241)
XbaI(3263)
SalI(3269)
HindIII(3287)

FIG. 9B

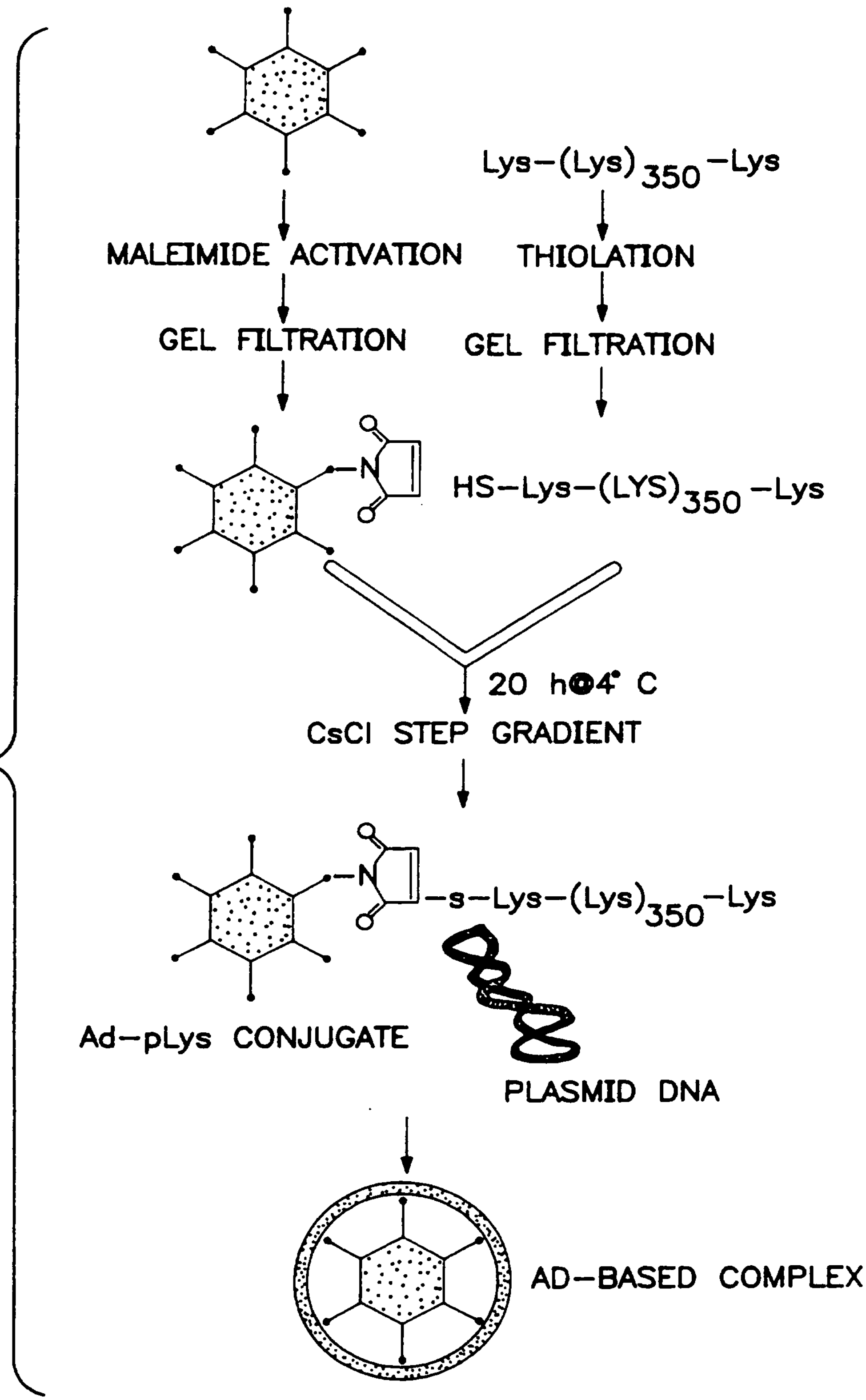
Lys–(Lys)350–Lys
MALEIMIDE ACTIVATION
THIOLATION
GEL FILTRATION
GEL FILTRATION
N
HS–Lys–(LYS)350–Lys
20 h@4° C
CsCl STEP GRADIENT
N
–s–Lys–(Lys)350–Lys
Ad–pLys CONJUGATE
PLASMID DNA
AD–BASED COMPLEX

FIG. 10

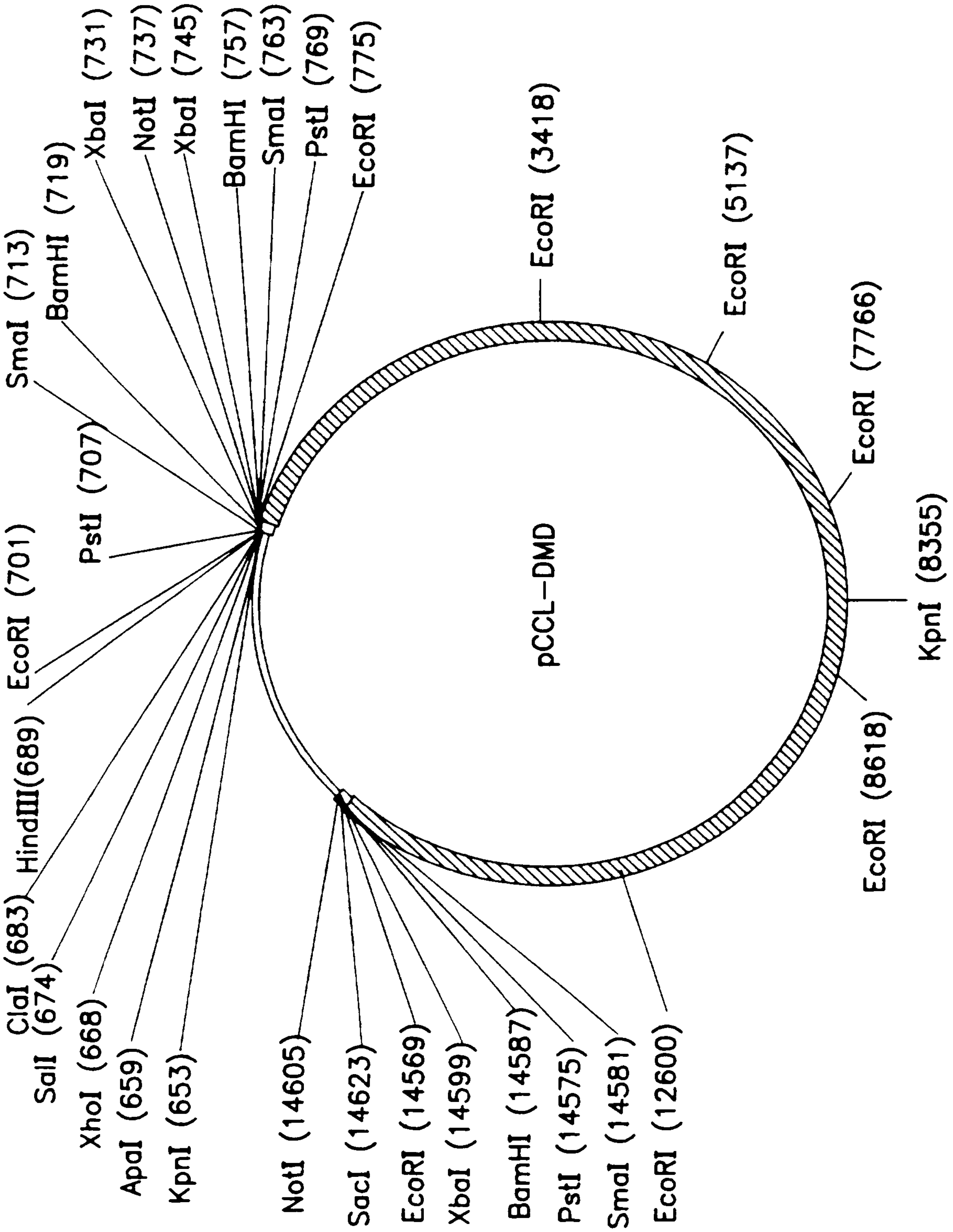
pCCL-DMD
XbaI (731)
NotI (737)
XbaI (745)
BamHI (757)
SmaI (763)
PstI (769)
EcoRI (775)
BamHI (719)
SmaI (713)
PstI (707)
EcoRI (701)
HindIII(689)
ClaI (683)
SalI (674)
XhoI (668)
ApaI (659)
KpnI (653)
EcoRI (3418)
EcoRI (5137)
EcoRI (7766)
KpnI (8355)
EcoRI (8618)
NotI (14605)
SacI (14623)
EcoRI (14569)
XbaI (14599)
BamHI (14587)
PstI (14575)
SmaI (14581)
EcoRI (12600)

FIG. 11

FIGURE 12A

CCAATTCCAT CATCAATAAT ATACCTTATT TTGGATTGAA GCCAATATGA 50
TAATGAGGGG GTGGAGTTTG TGACGTGGCG CGGGGCGTGG GAACGGGGCG 100
GGTGACGTAG GTTTTAGGGC GGAGTAACTT GTATGTGTTG GGAATTGTAG 150
TTTTCTTAAA ATGGGAAGTT ACGTAACGTG GGAAAACGGA AGTGACGATT 200
TGAGGAAGTT GTGGGTTTTT TGGCTTTCGT TTCTGGGCGT AGGTTCGCGT 250
GCGGTTTTCT GGGTGTTTTT TGTGGACTTT AACCGTTACG TCATTTTTTA 300
GTCCTATATA TACTCGCTCT GCACTTGGCC CTTTTTTACA CTGTGACTGA 350
TTGAGCTGGT GCCGTGTCGA GTGGTGTTTT TTTAATAGGT TTTCTTTTTT 400
ACTGGTAAGG CTGACTGTTA GGCTGCCGCT GTGAAGCGCT GTATGTTGTT 450
CTGGAGCGGG AGGGTGCTAT TTTGCCTAGG CAGGAGGGTT TTTCAGGTGT 500
TTATGTGTTT TTCTCTCCTA TTAATTTTGT TATACCTCCT ATGGGGGCTG 550
TAATGTTGTC TCTACGCCTG CGGGTATGTA TTCCCCCCAA GCTTGCATGC 600
CTGCAGGTCG ACTCTAGAGG ATCCGAAAAA ACCTCCCACA CCTCCCCCTG 650
AACCTGAAAC ATAAAATGAA TGCAATTGTT GTTGTTAACT TGTTTATTGC 700
AGCTTATAAT GGTTACAAAT AAAGCAATAG CATCACAAAT TTCACAAATA 750
AAGCATTTTT TTCACTGCAT TCTAGTTGTG GTTTGTCCAA ACTCATCAAT 800
GTATCTTATC ATGTCTGGAT CCCCGCGGCC GCTCTAGAAC TAGTGGATCC 850
CCCGGGCTGC AGGAATTCCG TAACATAACT GCGTGCTTTA TTGAGATACA 900
CAGTAAAGCA GTAATATAAT ACAATAGTAA GGCATATATT TGGTGAAATC 950
TGATATGTTG TGAAAATGCA GTAAAACTGA AGTTTAAAAA AATAATTAGT 1000
AAATGTTACA GTGTTGGTGT TAAAACACAA TCTATTATGA TACTCAAGTA 1050
AGAGTCCAGT ACCTGGAGAC AATGATGATA CATGCCATGT GATGATTATG 1100
CTTCAGTTAC ACTGATTATG ATTTACACTT TAATACTTGA TGGTTATAAA 1150
GAACATGAAA TGATGTCCAA ATTATGCTTA AAATCAGCAA TAAAGCTCTC 1200
AGTTTTTATT CAAATATTTT GATAGATTCA CTCCAGAACT AATATCTAAA 1250

FIGURE 12B

AGATAAAACG AAAAGATTAA AACAAAACTA TGCACTCTAT CTACCTTGGA 1300
TTTTAGAATG AAACTTAAAA CTTCTTAGTA GGAAAGGAAC CCCTTGTTTT 1350
AAATCTTGGT GAAAACAAAT CCTTGGATAA AGAAAATGCC CAGTGCCACA 1400
TAAAGGAGAG AGAGAGAGAA AAGCAAGACC AGAACCAAAT TTCAATTTGT 1450
TATCTTAGAG CTTTGGGTTT TCTTTTGGAA ATTATAAATG AAAAAAGGAA 1500
ACTGGTGTCC ACACAACAGA CAAGTGGTGA AGTTGTGAAA TTAGGTGTGC 1550
ACAATTACTA GAAACACCCC AAAACCAAAG TGAGGTAGAA ATAGCATGAG 1600
AAGCTGTGTT TGATGTTAAT TACAATTAAT AATGGACAAA ACCCACTCGC 1650
TAGAAGTTAA TTACACTTGA CGTTAGAGGT AACAGATTTG CAAAATGATA 1700
GGACAGTGAT TTCTATTGAG AGAATGCTCT TTAAATGCTA AGAAGAAGAA 1750
ACTGGCATGA GAGGAGTAAA GCTCTTCCTA GCAGTCCTTA GCTTTCTGTT 1800
GCACTTTTTC TCCTGGTTCA ATGACTTGCA TTTGTTTAGA CATTTCAGCC 1850
CGTCAACTAG ACCAGAGAGT TTGGAGACGC TTTTGCTCTC AAAACTTTCC 1900
AACCACTGTG CCTTCTCACC CACAATCCTG TGTGGAGTTA CTTGCAGGGA 1950
AACCAATGCA AAGGAGACAA ATGCAGTTCA TGGGCTTCTG GACTGATATT 2000
CACCAGGGTC ACAATGTGAT TGGGTTACTT TCTTAACAGT AATCCTAAGT 2050
CTTGCAGCAT TAAAAAAAAA AATCATCACA ATGAAGAAAA AAAAACCCAA 2100
AAAATCTAAA ATCTAAAATT CATCATCATC ATCAACAACA ACAACAACAA 2150
CAACAACAAA ACCACCCACT TCAGGTTGAG TTTATGAAGA GGGCAGAACA 2200
ATTTAGTTGT AATTATAGAG ATGTTTATAT GTATAGTTGT AAATATTCAT 2250
CCATTCTTTT ACAGAGTTGT TGCTCCCCTC ATATAAATTG ACTGAGGAGC 2300
CGCAACCTTT AGCTCCTACC ATCTTCCTCC TACTGTCTGG GAGTTAAAAA 2350
TGTCATCTGA TGTTCTATTG CAGAAACATC ATTAAATATA ACCCAACAGT 2400
AGGAAGTTGA ATATATCAGC CAACAAATTA CTATGATAGT AAGTCCTGTG 2450
TATTCATTCG CATGTTCCTT GAAAAAAATG AATCCTCTAG CTCTCAGTGG 2500

FIGURE 12C

AAAGTTTAAA ACTAGAAACA TCTGGAGCCC TAGACAATAT TTTAGTGTGG 2550
CGGTAGTCTC CTGGCTTTGG GCTCCAGGGA AAATTCACTC TTGCCCAAGC 2600
AGATAAGCCC AGATGACTAG AAGCAATTTC CATTAGGAAG TGGCAAGAAC 2650
ATTTGAAGAA GTAACTTCAT ATCTATTTAT CTATATACCT ATAGTATTTA 2700
TATACTTGTA GACATATAGA TGTATAAAAT GAAAGCCCAT AGCCAGCCCC 2750
ACTCAGTCAA CAATTCTCAA AAGAGCAATA TGAAGCAGTC ATTTGGTGGG 2800
GTTCGTATGC AAGAAAATAA AAAAACGTCA TGAATTCCAT ATGAATACCA 2850
CGCTAAAGTA ATGCAAAACA ATGTGCTGCC TCAGTGTGTG TGTGTGTGTG 2900
TGTGTGTGTG GTGGGTTCGT GCATGTATGT GTGCGTGTGT GTGTGTGTGT 2950
GTGTGTGTGT GTGTGTGTGC GTGTGTGTTT GTTTAGGGGT TTTTATAAAC 3000
AACTTTTTTT ATAAAGCACA CTTTAGTTTA CAATCTCTCT TTATAACTGT 3050
TATAAATTTT TAAACAACCC AAAATGCGTT CCATATAAAG AAATGGCAAG 3100
TTATTTAGCT ATCAAGATTT TACATGTTTT CTTTTAACTT TTTTGTACAA 3150
TTGCATAGAC GTGTAAAACC TGCCATTGTT AACAAAACAA TAACAGACTT 3200
AGAAACTACT GAAATCTACA GTATAGTACC ACTACCCTTC ACAAAAATAT 3250
AGATTTTATT TCTTGTAAAC TCTTACTGTC TAATCCTCTT TGTTGTACGA 3300
ATATTATAAA AACCATGCGG GAATCAGGAG TTGTAAAACA TTTATTCTGC 3350
TCCTTCTTCA TCTGTCATGA CTGAAACTAA GGACTCCATC GCTCTGCCCA 3400
AATCATCTGC CATGTGGAAA AGGCTTCCTA CATTGTGTCC TCTCTCATTG 3450
GCTTTCCGGG GGCATTTCTT CCTCTTGAAC TAGGGAAGGA GTTGTTGAGT 3500
TGCTCCATCA CTTCTTCTAA CCCTGTGCTT GTGTCCTGGG GAGGACTCAG 3550
AAGATCTTCC TCACCCATAG ATTCTGAAGT TTGACTGCCA ACCACTCGGA 3600
GCAGCATAGG CTGACTGCTA TCTGACCTCT GCAGAGAGGT GGAAGGAGAG 3650
GACACCGTGG TGCCATTCAC CTTAGCTTCA GCCTGGGGCT GCTCCAGGAG 3700
CTGTCTCAGT CTATGTAACT GAGACTCCAG CTGTTTATTG TGGTCTTCCA 3750

FIGURE 12D

GGATTTGCAT CCTGGCTTCC AGGCGTCCTT TGTGTTGGCG CAGTAGCTTA 3800
GCCTCAGCAA TGAGCTCAGC ATCCCTGGGA CTCTGAGGAG AGGTGGGCAT 3850
CATCTCAGGA GGAGATGGCA GTGGAGACAG GCCTTTATGC TCATGCTGCT 3900
GCTTCAGGCG ATCATATTCT GCTTGCAGAT TCCTGTTTTC TTCCTCAAGA 3950
TCTGCTAGGA TTCTCTCTAG CTCCCCTCTT TCCTCACTCT CTAAGGAAAT 4000
CAAGATCTGG GCAGGACTAC GAGGCTGGCT CAGGGGGGAG TCCTGGTTCA 4050
AACTTTGGCA GTAATGCTGG ATTAACAAAT GTTCATCATC TATGCTCTCA 4100
TTAGGAGAGA TGCTATCATT TAGATAAGAT CCATTGCTGT TTTCCATTTC 4150
TGCTAGCCTG CTAGCATAAT GTTCAATGCG TGAATGAGTA TCATCGTGTG 4200
AAAGCTGGGG GGACGAGGCA GGCGCAGAAT CTACTGGCCA GAAGTTGATC 4250
AGAGTAACGG GAGTTTCCAT GTTGTCCCCC TCTAACACAG TCTGCACTGG 4300
CAGGTAGCCC ATTCGGGGAT GCTTCGCAAA ATACCTTTTG GTTCGAAATT 4350
TGTTTTTTAG TACCTTGGCG AAGTCGCGAA CATCTTCTCC GGATGTAGTC 4400
GGAGTGCAAT ACTCTACCAT GGGGTAGTGC ATTTTATGGC CCTTTGCAAC 4450
TCGGCCAGAA AAAAAGCAAC TTTGGCAGAT GTCATAATTA AAATGCTTTA 4500
GGCTTCTGTA CCTGAATCCA ATGATTGGAC ACTCCTTACA GATGTTACAC 4550
TTGGCTTGAT GCTTGGCAGT TTCAGCAGCA GCCACTCTGT GCAAGACGGG 4600
CAGCCACACC ATAGACTGGG GTTCCAGGCG CATCCAGTCA AGGAAGAGAG 4650
CAGCTTCAAT CTCAGGTTTA TTATTGGCAA ATTGGAAGCA GCTCCTGACA 4700
CTCGGCTCAA TGTTACTGCC CCCAAAGGAA GCAACTTCAC CCAACTGTCT 4750
TGGGATTTGA ATAGAATCAT GCAGAAGAAG ACCCAGCCTA CGCTGGTCAC 4800
AAAAGCCAGT TGAACTTGCC ACTTGCTTGA AAAGGTATCT GTACTTGTCT 4850
TCCAAGTGTG CTTTACACAG AGAAATGATG CCAGTTTTAA AAGACAGGAC 4900
ACGGATCCTC CCTGTTCGTC CCGTATCATA AACATTGAGA AGCCAGTTGA 4950
GACACATATC CACACAGAGA GGGACATTGA CCAGATTGTT GTGCTCTTGC 5000
TCCAGACGAT CATAAATTGT AGTCAAACAG TTAATTATCT GCAGGATATC 5050

FIGURE 12E

CATGGGCTGG	TCATTTTGCT	TGAGGTTGTG	CTGGTCCAGG	GCATCACATG	5100
CAGCTGACAG	GCTCAAGAGA	TCCAAGCAAA	GGGCCTTCTG	GAGCCTTCTG	5150
AGCTTCATGG	CAGTCCTATA	CGCGGAGAAC	CTGACATTAT	TCAGGTCAGC	5200
TAAAGACTGG	TAGAGCTCTG	TCATTTTGGG	GTGGTCCCAA	CAAGTGGTTT	5250
GGGTCTCGTG	GTTGATATAG	TAGGGCACTT	TGTTTGGTGA	GATGGCTCTC	5300
TCCCAGGGAC	CCTGAACTGA	AGTGGAAAGG	AAGTGCTGGG	ATGCAGGACC	5350
AAAGTCCCTG	TGGGCTTCAT	GCAGCTGTCT	GACACGGTCC	TCCACAGCCA	5400
CCTGTAGAAG	CCTCCATCTG	GTATTCAGAT	CTTCCAAAGT	GCTGAGGTTA	5450
TAAGGTGAGA	GCTGAATGCC	CAGTGTGGTC	AGCTGATGTG	CAAGGTCATT	5500
GACACGATTG	ACATTCTCTT	TAAGAGGTGC	AATTTCTCCC	CGAAGTGCCT	5550
TGACTTTTTC	AAGGTGATCT	TGCAGAGAGT	CAATGAGGAG	ATCCCCCACT	5600
GGCTGCCAGG	ATCCCTTGAT	CACCTCAGCT	TGGCGCAACT	TGAGGTCCAG	5650
TTCATCGGCA	GCTTCCTGAA	GTTCCTGGAG	TCTTTCAAGA	GCTTCATCTA	5700
TTTTTCTCTG	CCAATCAGCT	GAGCGCAGGT	TCAATTTGTC	CCATTCAGCG	5750
TTGACCTCTT	CAGCCTGCTT	TCGTAGGAGC	CGAGTGACAT	TCTGAGCTCT	5800
TTCTTCAGGA	GGCAGTTCTC	TGGGCTCCTG	GTAGAGTTTC	TCTAGTCCTT	5850
CCAAAGGCTG	CTCTGTCAGA	AATATTCTCA	CAGTCTCCAG	AGTACTCATG	5900
ATTACAGGTT	CTTTAGTTTT	CAATTCCCTC	TTGAAGGCCC	TATGTATATC	5950
ATTCTGCTTC	TGAACTGCTG	GGAAATCACC	ACCGATGGGT	GCCTGACGGC	6000
TCAGTTCATC	ATCTTTCAGC	TGTAGCCAAA	CAAGAAGTTC	CTGAAGAGAA	6050
AGATGCAAAC	GCTTCCACTG	GTCAGAACTT	GCTTCCAAAT	GGGACCTAAT	6100
GTTGAGAGAC	TTTTTCTGAA	GTTCACTCCA	CTTGAAATTC	ATGTTATCCA	6150
AACGTCTTTG	TAACAGGGGT	GCTTCATCCG	AACCTTCCAG	GGATCTCAGG	6200
ATTTTTTGGC	CATTTTCATC	AAGATTGTGA	TAGATATCTG	TGTGAGTTTC	6250
AATTTCTCCT	TGGAGATCTT	GCCATGGTTT	CATCAGCTCT	CTGACTCCCC	6300
TGGAGTCTTC	TAGGAGCTTC	TCCTTACGGG	AAGCGTCCTG	TAGGACATTG	6350

FIGURE 12F

GCAGTTGTTT CTGCTTCCGT AATCCAGGAA AGAAACTTCT CCAGGTCCAG 6400
AGGGAACTGC TGCAGTAATC TATGAGTTTC TTCCAAAGCA GCCTCTTGCT 6450
CACTTACTCT TTTATGAATG TTTCCCCAAG AAGTATTGAT ATTCTCTGTT 6500
ATCATGTGTA CTTTTCTGGT ATCATCAGCA GAATAGTCCC GAAGAAGTTT 6550
CAGTGCCAAA TCATTTGCCA CGTCTACACT TATCTGCCGT TGACGGAGGT 6600
CTTTGGCCAA CTGCTTGGTT TCTGTGATCT TCTTTTGGAT TGCATCTACT 6650
GTGTGAGGAC CTTCTTTCCA TGAGTCAAGC TTGCCTCTGA CCTGTCCTAT 6700
GACCTGTTCG GCTTCTTCCT TAGCTTCCAG CCATTGTGTT GAATCCTTTA 6750
ACATTTCATT CAACTGTTGT CTCCTGTTCT GCAGCTGTTC TTGAACCTCA 6800
TCCCACTGAA TCTGAATTCT TTCAATTCGA TCAGTAATGA TTGTTCTAGC 6850
TTCTTGATTG CTGGTTTTGT TTTTCAAATT CTGGGCAGCA GTAATGAGTT 6900
CTTCCAATTG GGGGCGTCTC TGTTCCAAAT CTTGCAGTGT TGCCTTCTGT 6950
TTGATGATCA TTTCATTGAT GTCTTCCAGA TCACCCACCA TCACTCTCTG 7000
TGATTTTATA ACTCGATCAA GCAGAGACAG CCAGTCTGTA AGTTCTGTCC 7050
AAGCTCGGTT GAAGTCTGCC AGTGCAGGTA CCTCCAACAG CAAAGAAGAT 7100
GGCATTTCTA GTTTGGAGAT GACAGTTTCC TTAGTAACCA CAGATTGTGT 7150
CACTAGAGTA ACAGTCTGAC TGGCAGAGGC TCCAGTAGTG CTCAGTCCAG 7200
GGGCACGGTC AGGCTGCTTT GTCCTCAGCT CCCGAAGTAA ATGGTTTACA 7250
GCCTCCCACT CAGACCTCAG ATCTTCTAAC TTCCTCTTCA CTGGCTGAGT 7300
GCTTGGTTTT TCCTTATACA AATGCTGCCC TTTCGACAAA AGCCTTTCCA 7350
CATCCGCTTG TTTACCGTGA ACTGTTACTT CAATCTCCTT TATGTCAAAC 7400
GGTCCTGCCT GACTTGGTTG GTTATAAATT TCCAACTGGT TTCTAATAGG 7450
AGAGACCCAC AGAAGCAGGT GATCCAGCTG CTCTTCAAGC TGCCTAAAAT 7500
CTTTTAAGTG AACCTCAAGC TCTCCTTGTT TCTCAGGTAA AGCTCTGGAG 7550
ACCTTTATCC ACTGGAGATT TGTCTGTTTG AGCTTCTTTT CAAGTTTATC 7600

FIGURE 12G

TTGCTCTTCT GGCCTTATGG GAGCACTTAC AAGTACTGCT CCTCCTGTTT 7650
CATTTAATTG TTTTAGAATT CCCTGGCGCA GGGGCAACTC TTCTGCCAGT 7700
AACTTGACTT GTTCAAGTTG TTCTTTTAGC TGCTGCTCAT CTCCAAGTGG 7750
AGTAATAGCA ATGTTATCTG CTTCTTCCAG CCACAAAACA AATTCATTTA 7800
AATCTCTTTG AAATTCTGAC AAGACATTCT TTTGTTCTTC AATCCTCTTT 7850
CTCCTTTCTG CCAGCTCTTT GCAGATGTCG TGCCACCGCA GACTCAAGCT 7900
TCCTAATTTT TCTTGTAGAA TATTGACATC TGTTTTTGAA GACTGTTGAA 7950
TTATTTCTTC CCCAGTTGCA TTCAGTGTTC TGACAACAGC TTGACGCTGC 8000
CCAATGCCAT CCTGGAGTTC CTTAAGATAC CATTTGTATT TAGCATGTTC 8050
CCAGTTTTCA GGATTTTGTG TCTTTTTGAA AAACTGTTCA ACTTCATTCA 8100
GCCATTGATT AAATACCTTC ATATCATAAT GAAAGTGTCG CCATTTTTCA 8150
ACTGATCTGT CGAATCGCCC TTGTCGTTCC TTGTACATTC TATGAAGTTT 8200
TTCCCCCTGG AAATCCATCT GTGCCACGGC TTCCTGTACT TTCACCTTTT 8250
CCATGGAGGT GGCACTTTGC AAGGCTGCTG TCTTCTTCTT GTGAATAATA 8300
TCAATCCGAC CTGAGATTTG TTGCAAATTG TCTTTTATAT TCTTAAGAGA 8350
CTCCTCTTGC TTAAAAAGAT CTTCAAAATC TTTAGCACAG AGTTCAGGAG 8400
TATTTAGAAG ATGATCAACT TCTGAAAGAG CTTGTAAGAT ATGACTGATC 8450
TCGGTCAAAT AAGTAGAAGG CACATAAGAA ACATCCAAAG GCATATCTTC 8500
AGTCGTCACT ACCATAGTTT CTTCATGGAG AGTGTGAATT TGTGCAAAGT 8550
TGAGTCTTCG AAACTGAGCA AAATTGCTCT CAATTTGCCG CCAGCGCTTG 8600
CTGAGCTGGA TCTGAGTTGG CTCCACTGCC ATTGCGGCCC CATTCTCAGA 8650
CAAGCCCTCA GCTTGCCTGC GCACTGCATT CAGCTCCTCT TTCTTCTTCT 8700
GCAATTCACG ATCAATTTCC TTTAATTTTC TTTCATCTCT GGGTTCAGGT 8750
AGGCTGGCTA ATTTTTTTTC AATTTCATCC AAGCATTTCA GGAGATCATC 8800
AGCCTGCCTC TTGTACTGAT ACCACTGGTG AGAAATTTCT AGGGCCTTTT 8850

FIGURE 12H

TTCTTCTTTG	AGACCTCAAA	TCCTTGAGAG	CATTATGTTT	TGTCTGTAAC	8900
AGCTGCTGTT	TTATCTTTAT	TTCCTCTCGC	TTTCTCTCAT	CTGTGATTCT	8950
TTGTTGTAAG	TTGTCTCCTC	TTTGCAACAA	TTCATTTACA	GTACCCTCAT	9000
TGTCTTCACT	CATATCTTTA	TTGAAGTCTT	CCTCTTTCAG	ATTCACCCCC	9050
TGCTGAATTT	CAGCCTCCAG	TGGTTCAAGC	AATTTTTGTA	TATCTGAGTT	9100
AAACTGCTCC	AATTCCTTCA	AAGGAATGGA	GGCCTTTCCA	GTCTTAATTC	9150
TGTGAGAAAT	AGCTGCAAAT	CGACGGTTGA	GCTCAGAGAT	TTGGGGCTCT	9200
ACTACTTTCC	TGCAGTGGTC	ACCGCGGTTT	GCCATCAATT	TTGCTGCTTG	9250
GTCACGTGTG	GAGTCCACCT	TTGGGCGCAT	GTCATTCATT	TCAGCCTTTA	9300
AACGCTTAAG	AATGTCTTCC	TTTTGTTGTG	GTTTCTTCTT	TTCAGACTCA	9350
TCTAAAAGTT	CATCTGCATG	AATGATCCAC	TTTGTGATTT	GTTCTATGTT	9400
CTGATCAAAG	GTTTCCATGT	GTTTCTGGTA	TTCCAACAAA	AGATTTAGCC	9450
ATTCTTCTAC	TCTGGAGGTG	ACAGCTATCC	AGTTACTGTT	CAGAAGACTC	9500
AGTTTATCTT	CTACCAAGGT	TTCTTTCTTG	CCCAACACCA	TTTTCAAAGA	9550
CTCTCCTAAT	TCTGTAACAC	TCTTCAAGTG	AGCCTTCTGT	TTCTCAATCT	9600
CTTTTTGAGT	AGCCTTTCCC	CAGGCAACTT	CAGAATCCAA	ATTACTTGGC	9650
ATTCCTTCAA	CTGCTGATCT	CTTCGTCAAT	TCTGTATCTG	TTGCTGCCAG	9700
CCATTCTGTT	AAGACATTCA	TTTCCTTTCT	CATCTTACGG	GACAACTTCA	9750
AGCATTTCTC	CAACTGTTGC	TTTCTCTCTG	TTACCTTCGC	ACCCAACTCA	9800
TTGTAATGCA	ATTTCAAAGC	TGTTACTCGT	TCATCAAGCT	CTTTGGGATT	9850
TTCTGTCTGC	TTTTTCTGTA	CAATTTGACG	TCCGGTTTTA	ATCACCATTT	9900
CCACTTCAGA	CTTGACTTCA	CTCAGGCTTT	TATACAAGTT	CACACAATGA	9950
CTTAGTTGTG	ACTGAATTAC	TTCCTGTTCA	ACACTCTTGG	TTTCCAATGC	10000
AGGCAAATGC	ATCTTGACTT	CATCTAAAAT	CATCTTACTT	TCCTCTAGAC	10050
GTTGTTCAAA	ATTGGCTGGT	TTTTGGAATA	ATCGAAATTT	CATGGAGACA	10100
TCTTGTAATT	TTTTCTGTGC	AACATCAATT	TGTGAAAGAA	CCCTTTGGTT	10150

FIGURE 12I

GGCATCCTTC CCCTGGTTAT GTTTCTTCAT TTCTTCTAAA CTTATCTCAT 10200
GACTTGTCAA ATCTGATTGG ATTTTCTGGG CTTCCTGAGG CATTTGAGCT 10250
GCATCCACCT TGTCAGTGAT ATAAGCTGCC AACTGCTTGT CAATGAATTC 10300
AAGCGACTCC TGAATTAAGT GCAAGGACTT TTCAATTTCC TGGGCAGACT 10350
GGATACTCTG TTCAAGCAAC TTTTGTTTCC TCACAGCCTC TTCATGTAGT 10400
TCCCTCCAAC GAGAATTAAA CGTCTCAAGC TCCTCATTGA TCAGTTCATC 10450
CATGACTCCT CCATCTGTAA GAGTCTGTGC CAATAGACGA ATCTGATTTG 10500
GGTTCTCCTC TGAATGATGC ATCAGATTTT CAAGAGATTC TAGCACTTCA 10550
GTGATTTCCT CAGGTCCTGC AGGAACATTT TCCATGGTTT TAAGTTTCAA 10600
TTCTACTTCA TTGAGCCACT TGTTTGCTTT CTCTAAATAT GACAATAACT 10650
CATGCCAACA TGCCCAAACT TCTTCCAAAG TTTTGCATTT TCCATTCAGC 10700
CTGGTGCACA GCCATTGGTA GTTGGTGGTC AGAGTTTCAA GTTCCTTTTT 10750
TAAGGCCTCT TGTGCTGAGG GTGGAGCGTG AGCTATTACA CTATTTACAG 10800
TCTCAGTAAG GAGTTTCACT TTAGTTTCTT TTTGTAGTGC CTCTTCTTTA 10850
GCTCTCTTCA TTTCTTCAAC AGCAGTCTGT AATTCATCTG GAGTTTTATA 10900
TTCAAAATCT CTCTCTAGAT ATTCTTCTTC AGCTTGTGTC ATCCACTCAT 10950
GCATCTCTGA TAGATCTTTT TGGAGGCTTA CGGTTTTATC CAAACCTGCC 11000
TTTAAGGCTT CCTTTCTGGT GTAGACCTGG CGGCATATGT GATCCCACTG 11050
AGTGTTAAGC TCTCTAAGTT CTGTCTCCAG TCTGGATGCA AACTCAAGTT 11100
CAGCTTCACT CTTTATCTTC TGCCCACCTT CATTAACACT ATTTAAACTG 11150
GGCTGAATTG TTTGAATATC ACCAACTAAA AGTCTGCATT GTTTGAGCTG 11200
TTTTTTCAGG ATTTCAGCAT CCCCCAGGGC AGGCCATTCC TCTTTCAGGA 11250
AAACATCAAC TTCAGCCATC CATTTCTGTA AGGTTTTTAT GTGATTCTGA 11300
AATTTTCGAA GTTTATTCAT ATGTTCTTCT AGCTTTTGGC AGCTTTCCAC 11350
CAACTGGGAG GAAAGTTTCT TCCAGTGCCC CTCAATCTCT TCAAATTCTG 11400

FIGURE 12J

ACAGATATTT CTGGCATATT TCTGAAGGTG CTTTCTTGGC CATCTCCTTC 11450

ACAGTGTCAC TCAGATAGTT GAAGCCATTT TGTTGCTCTT TCAAAGAACT 11500

TTGCAGAGCC TGTAATTTCC CGAGTCTCTC CTCCATTATT TCATATTCAG 11550

TAACACTAAG ATAAGGTACA GAGAGTTTGC TTTCTGACTG CTGGATCCAC 11600

GTCCTGATGC TACTCATTGT CTCCTGATAG CGCATTGGTG GTAAAGTGTC 11650

AAAAATTGTC TGTAGCTCTT TCTCTTTGGC CCTCACACCA TCAAAGATGT 11700

GGTTAAAATG ATTAGTAAAG GCCACAAAGT CTGCATCCAG AAACATTGGC 11750

CCCTGTCCCT TTTCTTTCAG TTGTAGACTC TGAATTTTTA ATTGCTCAAT 11800

TTGAGGCTGA AGAGCTGACA ATCTGTTGAC TTCATCCTTA CAAATTTTTA 11850

ACTGGCTTTT AATTGCTGTT GGCTCTGATA GGGTGGTAGA CTGGGTTTTC 11900

AACAAGTTTT CGGCAGTAGT TGTCATCTGT TCCAATTGTT GTAGCTGATT 11950

ATAAAAGGTA ATGATGTTGG TTTGATACTC TAGCCAGTTA ACTCTCTCAC 12000

TCAGCAATTG GCAGAATTCT GTCCACCGGC TGTTCAGTTG TTCTGAAGCT 12050

TGTCTGATAC TTTCAGCATT AACACCCTCA TTTGCCATCT GTTCCACCAG 12100

GGCCTGAGCT GATCTGCTGG CATCTTGCAG TTTTCTGAAC TTCTCTGCTT 12150

TTTCTCGTGC TATGGCATTG ACTTTTTCTT GCAAGTCTGA GATGTTGCCT 12200

TCTTTTCGAT AGACTGCAAA TTCAGAACTC TGTAATACAG CTTCTGAACG 12250

AGTAATCCAA CTGTGAAGTT CAGTTATATC GACATCCAAC CTTTTCCTGA 12300

GTTCAGAATC CACAGTTATC TGCCTCTTCT TTTGAGGAGG TGGTGGTGGA 12350

AGTTCCTCTT GGGCATGTTT TACCATGATT TGTTCCCTTG TGGTCACCAT 12400

AGTTACCGTT TCCATTACAG TTGTCTGTGT TAGGGATGGT TGAGTGGTGG 12450

TGACAGCCTG TGAAATTTGT GCTGAACTCT TTTCAAGTTT TTGGGTTAAA 12500

TTGTCCCAAC GTTGTGCAAA GTTTTCCATC CAGATTTCCA TCTTTTGAGT 12550

CACTGACTTA TTTTTCAGTG CCGAAAGTAG ATCTTGATTG AGTGAACTTA 12600

GTTTTTCCAT GGTTGGCTTT TTCTTTTCTA GATCTATTTT TAAAGTAGAT 12650

FIGURE 12K

ATTTTGTGAA GACTTGACAT CATTTCATTT TGATCTTTAA AGCCACTTGT 12700
CTGAATGTTC TTCATTGCAT CTTCTTTTTC TGAAAGCCAT GTACTAAAAA 12750
GGCACTGTTC TTCAGTAAAA TGCTGCCATT TTAGAAGAAT ATCTTGTAAA 12800
ACAATCCAGC GGTCTTCAGT CCATCTGCAG ATATTTGCCC ATCGATCTCC 12850
CAGTACCTTA AGTTGTTCTT CCAAAGCAGC TGTTGCATGA TCACCGCTGG 12900
ATTCATCAAC CACTACTACC ATGTGAGTGA GCGAGTTGAC CCTGACCTGC 12950
TCCTGTTCTA GATCTTCTTG AAGCACCTTA TGTTGTTGTA CTTGGCATTT 13000
TAGATCTTCA AGATCAGGTC CAAAGGGCTC TTCCTCCATT TTCTTAGTTC 13050
TCTCTTCAGT TTTTGTTAAC CAGTCATCTA GTTCTTTTAA TTTCTGATTC 13100
TGGAGATCCA TTAGAACTTT GTGTAATTTG CTTTGTTTTT CCATGCTAGC 13150
TACCCTGAGA CATTCCCATC TTGAATTTAG GAGATTCATT TGTTCTTGCA 13200
CTTCAGCTTC TTCATCTTCT GATAATTTCC CTTTTCCAAC TAGTTGACTT 13250
CCTAACTGTA GAACATTACC AACAAGTCCT TGATGAGATG TCAGATCCAT 13300
CATGAATCCC TCATGAGCAT GAAACTGTTC TTTCACTTCT TCAACATCAT 13350
TTGAAATCTC TCCTTGTGCT CGCAATGTAT CCTCGGCAGA AAGAAGCCAT 13400
GAAAGTACTT CTTCTAAAGC AGTTTGGTAA CTATCCAGAT TTACTTCCGT 13450
CTCCATCAAT GAACTGTCAA GTGACTTGTC TCTGGGAGCT TCCAAATGCT 13500
GTGAAGGATA GGGGCTCTGT GTGGAATCAG AGGTGGCAAC ATAAGCAGCC 13550
TGTGTGAAGG CATAACTCTT GAATCGAGGC TTAGGAGATG AAGAAGTTTG 13600
TTCATAGCCC TGTGCTAGAC TGACTGTGAT CTGTTGAGAG TAATGCATCT 13650
GGTGATGTAA TTGAAAATGT TCTTCTCTAG TTACTTTTGA AGATGTCCTG 13700
GGCAACATTT CCACTTCTTG AATGGCTTCA ATGCTCACTT GTTGTGGCAA 13750
AACTTGAAAG AGTGATGTGA TGTACATTAA GATGGACTTC TTGTCTGGAT 13800
AAGTGGTAGC AACATCTTCA GGATCAAGAA GTTTTTCTAT GCCTAACTGG 13850
CATTTTGCAA TGTTGAAGGC ATGTTCCAGT CTTTGGGTGG CTGAGTGCTG 13900

FIGURE 12L

TGAAACCACA CTATTCCAAT CAAACAGGTC GGGCCTGTGA CTATGGATAA 13950
GAGCATTCAA AGCCAACCCG TCGGACCAGC TAGAGGTGAA GTTGATGACG 14000
TTAACCTGTG GATAATTACG TGTTGACTGT CGAACCCAGC TCAGAAGAAT 14050
CTTTTCACTG TTGGTTTGCT GCAATCCAGC CATGATAGTT TTCATCACAT 14100
TTTTGACCTG CCAGTGGAGG ATTATATTCC AAATCAAACC AAGAGTGAGT 14150
TTATGATTTC CATCCACTAT GTCAGTGCTT CCTATATTCA CTAAATCAAC 14200
ATTATTTTTC TGTAAGACCC GCAGTGCCTT GTTGACATTG TTCAGGGCAT 14250
GAACTCTTGT AGATCCCTTT TCTTTTGGCA GTTTTTGCCC TGTAAGGCCT 14300
TCCAAGAGGT CTAGGAGGCG TTTTCCATCC TGCAGGTCAC TGAAGAGGTT 14350
GTCTATGTGT TGCTTTCCAA ACTTAGAAAA TTGTGCATTT ATCCATTTTG 14400
TGAATGTTTT CTTTTGAACA TCTTCTCTTT CATAACAGTC CTCTACTTCT 14450
TCCCACCAAA GCATTTGGAA GAAAAAGTAT ATATCAAGGC AGGGATAAAA 14500
ATCTTGGTAA AAGTTTCTCC CAGTTTTATT GCTCCAGGAG GCTTAGGTAC 14550
GATGAGAAGC CAATAAACTT CAGCAGCCTT GACAAAAAAA AAAAAAAAAA 14600
TAGCACTTCA AGTCTTCCTA TTCGTTTTTT CTATAAAGCT ATTGCCTTCA 14650
AGAGCGGAAT TCCTGCAGCC CGGGGGATCC ACTAGTTCTA GAGCGGCCGC 14700
GGGTACAATT CCGCAGCTTT TAGAGCAGAA GTAACACTTC CGTACAGGCC 14750
TAGAAGTAAA GGCAACATCC ACTGAGGAGC AGTTCTTTGA TTTGCACCAC 14800
CACCGGATCC GGGACCTGAA ATAAAAGACA AAAAGACTAA ACTTACCAGT 14850
TAACTTTCTG GTTTTTCAGT TCCTCGAGTA CCGGATCCTC TAGAGTCCGG 14900
AGGCTGGATC GGTCCCGGTG TCTTCTATGG AGGTCAAAAC AGCGTGGATG 14950
GCGTCTCCAG GCGATCTGAC GGTTCACTAA ACGAGCTCTG CTTATATAGA 15000
CCTCCCACCG TACACGCCTA CCGCCCATTT GCGTCAATGG GGCGGAGTTG 15050
TTACGACATT TTGGAAAGTC CCGTTGATTT TGGTGCCAAA ACAAACTCCC 15100
ATTGACGTCA ATGGGGTGGA GACTTGGAAA TCCCCGTGAG TCAAACCGCT 15150
ATCCACGCCC ATTGATGTAC TGCCAAAACC GCATCACCAT GGTAATAGCG 15200

FIGURE 12M

ATGACTAATA	CGTAGATGTA	CTGCCAAGTA	GGAAAGTCCC	ATAAGGTCAT	15250
GTACTGGGCA	TAATGCCAGG	CGGGCCATTT	ACCGTCATTG	ACGTCAATAG	15300
GGGGCGTACT	TGGCATATGA	TACACTTGAT	GTACTGCCAA	GTGGGCAGTT	15350
TACCGTAAAT	ACTCCACCCA	TTGACGTCAA	TGGAAAGTCC	CTATTGGCGT	15400
TACTATGGGA	ACATACGTCA	TTATTGACGT	CAATGGGCGG	GGGTCGTTGG	15450
GCGGTCAGCC	AGGCGGGCCA	TTTACCGTAA	GTTATGTAAC	GACCTGCAGG	15500
TCGACTCTAG	AGGATCTCCC	TAGACAAATA	TTACGCGCTA	TGAGTAACAC	15550
AAAATTATTC	AGATTTCACT	TCCTCTTATT	CAGTTTTCCC	GCGAAAATGG	15600
CCAAATCTTA	CTCGGTTACG	CCCAAATTTA	CTACAACATC	CGCCTAAAAC	15650
CGCGCGAAAA	TTGTCACTTC	CTGTGTACAC	CGGCGCACAC	CAAAAACGTC	15700
ACTTTTGCCA	CATCCGTCGC	TTACATGTGT	TCCGCCACAC	TTGCAACATC	15750
ACACTTCCGC	CACACTACTA	CGTCACCCGC	CCCGTTCCCA	CGCCCCGCGC	15800
CACGTCACAA	ACTCCACCCC	CTCATTATCA	TATTGGCTTC	AATCCAAAAT	15850
AAGGTATATT	ATTGATGATG	CTAGCGGGGC	CCTATATATG	GATCCAATTG	15900
CAATGATCAT	CATGACAGAT	CTGCGCGCGA	TCGATATCAG	CGCTTTAAAT	15950
TTGCGCATGC	TAGCTATAGT	TCTAGAGGTA	CCGGTTGTTA	ACGTTAGCCG	16000
GCTACGTATA	CTCCGGAATA	TTAATAGGCC	TAGGATGCAT	ATGGCGGCCG	16050
GCCGCCTGCA	GCTGGCGCCA	TCGATACGCG	TACGTCGCGA	CCGCGGACAT	16100
GTACAGAGCT	CGAGAAGTAC	TAGTGGCCAC	GTGGGCCGTG	CACCTTAAGC	16150
TTGGCACTGG	CCGTCGTTTT	ACAACGTCGT	GACTGGGAAA	ACCCTGGCGT	16200
TACCCAACTT	AATCGCCTTG	CAGCACATCC	CCCTTTCGCC	AGCTGGCGTA	16250
ATAGCGAAGA	GGCCCGCACC	GATCGCCCTT	CCCAACAGTT	GCGCAGCCTG	16300
AATGGCGAAT	GGCGCCTGAT	GCGGTATTTT	CTCCTTACGC	ATCTGTGCGG	16350
TATTTCACAC	CGCATACGTC	AAAGCAACCA	TAGTACGCGC	CCTGTAGCGG	16400
CGCATTAAGC	GCGGCGGGTG	TGGTGGTTAC	GCGCAGCGTG	ACCGCTACAC	16450

FIGURE 12N

```
TTGCCAGCGC CCTAGCGCCC GCTCCTTTCG CTTTCTTCCC TTCCTTTCTC   16500
GCCACGTTCG CCGGCTTTCC CCGTCAAGCT CTAAATCGGG GGCTCCCTTT   16550
AGGGTTCCGA TTTAGTGCTT TACGGCACCT CGACCCCAAA AAACTTGATT   16600
TGGGTGATGG TTCACGTAGT GGGCCATCGC CCTGATAGAC GGTTTTTCGC   16650
CCTTTGACGT TGGAGTCCAC GTTCTTTAAT AGTGGACTCT TGTTCCAAAC   16700
TGGAACAACA CTCAACCCTA TCTCGGGCTA TTCTTTTGAT TTATAAGGGA   16750
TTTTGCCGAT TTCGGCCTAT TGGTTAAAAA ATGAGCTGAT TTAACAAAAA   16800
TTTAACGCGA ATTTTAACAA AATATTAACG TTTACAATTT TATGGTGCAC   16850
TCTCAGTACA ATCTGCTCTG ATGCCGCATA GTTAAGCCAG CCCCGACACC   16900
CGCCAACACC CGCTGACGCG CCCTGACGGG CTTGTCTGCT CCCGGCATCC   16950
GCTTACAGAC AAGCTGTGAC CGTCTCCGGG AGCTGCATGT GTCAGAGGTT   17000
TTCACCGTCA TCACCGAAAC GCGCGAGACG AAAGGGCCTC GTGATACGCC   17050
TATTTTTATA GGTTAATGTC ATGATAATAA TGGTTTCTTA GACGTCAGGT   17100
GGCACTTTTC GGGGAAATGT GCGCGGAACC CCTATTTGTT TATTTTTCTA   17150
AATACATTCA AATATGTATC CGCTCATGAG ACAATAACCC TGATAAATGC   17200
TTCAATAATA TTGAAAAAGG AAGAGTATGA GTATTCAACA TTTCCGTGTC   17250
GCCCTTATTC CCTTTTTTGC GGCATTTTGC CTTCCTGTTT TTGCTCACCC   17300
AGAAACGCTG GTGAAAGTAA AAGATGCTGA AGATCAGTTG GGTGCACGAG   17350
TGGGTTACAT CGAACTGGAT CTCAACAGCG GTAAGATCCT TGAGAGTTTT   17400
CGCCCCGAAG AACGTTTTCC AATGATGAGC ACTTTTAAAG TTCTGCTATG   17450
TGGCGCGGTA TTATCCCGTA TTGACGCCGG GCAAGAGCAA CTCGGTCGCC   17500
GCATACACTA TTCTCAGAAT GACTTGGTTG AGTACTCACC AGTCACAGAA   17550
AAGCATCTTA CGGATGGCAT GACAGTAAGA GAATTATGCA GTGCTGCCAT   17600
AACCATGAGT GATAACACTG CGGCCAACTT ACTTCTGACA ACGATCGGAG   17650
GACCGAAGGA GCTAACCGCT TTTTTGCACA ACATGGGGGA TCATGTAACT   17700
```

FIGURE 120

CGCCTTGATC GTTGGGAACC GGAGCTGAAT GAAGCCATAC CAAACGACGA 17750
GCGTGACACC ACGATGCCTG TAGCAATGGC AACAACGTTG CGCAAACTAT 17800
TAACTGGCGA ACTACTTACT CTAGCTTCCC GGCAACAATT AATAGACTGG 17850
ATGGAGGCGG ATAAAGTTGC AGGACCACTT CTGCGCTCGG CCCTTCCGGC 17900
TGGCTGGTTT ATTGCTGATA AATCTGGAGC CGGTGAGCGT GGGTCTCGCG 17950
GTATCATTGC AGCACTGGGG CCAGATGGTA AGCCCTCCCG TATCGTAGTT 18000
ATCTACACGA CGGGGAGTCA GGCAACTATG GATGAACGAA ATAGACAGAT 18050
CGCTGAGATA GGTGCCTCAC TGATTAAGCA TTGGTAACTG TCAGACCAAG 18100
TTTACTCATA TATACTTTAG ATTGATTTAA AACTTCATTT TTAATTTAAA 18150
AGGATCTAGG TGAAGATCCT TTTTGATAAT CTCATGACCA AAATCCCTTA 18200
ACGTGAGTTT TCGTTCCACT GAGCGTCAGA CCCCGTAGAA AAGATCAAAG 18250
GATCTTCTTG AGATCCTTTT TTTCTGCGCG TAATCTGCTG CTTGCAAACA 18300
AAAAAACCAC CGCTACCAGC GGTGGTTTGT TTGCCGGATC AAGAGCTACC 18350
AACTCTTTTT CCGAAGGTAA CTGGCTTCAG CAGAGCGCAG ATACCAAATA 18400
CTGTTCTTCT AGTGTAGCCG TAGTTAGGCC ACCACTTCAA GAACTCTGTA 18450
GCACCGCCTA CATACCTCGC TCTGCTAATC CTGTTACCAG TGGCTGCTGC 18500
CAGTGGCGAT AAGTCGTGTC TTACCGGGTT GGACTCAAGA CGATAGTTAC 18550
CGGATAAGGC GCAGCGGTCG GGCTGAACGG GGGGTTCGTG CACACAGCCC 18600
AGCTTGGAGC GAACGACCTA CACCGAACTG AGATACCTAC AGCGTGAGCT 18650
ATGAGAAAGC GCCACGCTTC CCGAAGGGAG AAAGGCGGAC AGGTATCCGG 18700
TAAGCGGCAG GGTCGGAACA GGAGAGCGCA CGAGGGAGCT TCCAGGGGGA 18750
AACGCCTGGT ATCTTTATAG TCCTGTCGGG TTTCGCCACC TCTGACTTGA 18800
GCGTCGATTT TTGTGATGCT CGTCAGGGGG GCGGAGCCTA TGGAAAAACG 18850
CCAGCAACGC GGCCTTTTTA CGGTTCCTGG CCTTTTGCTG GCCTTTTGCT 18900
CACATGTTCT TTCCTGCGTT ATCCCCTGAT TCTGTGGATA ACCGTATTAC 18950

FIGURE 12P

CGCCTTTGAG TGAGCTGATA CCGCTCGCCG CAGCCGAACG ACCGAGCGCA 19000

GCGAGTCAGT GAGCGAGGAA GCGGAAGAGC GCCCAATACG CAAACCGCCT 19050

CTCCCCGCGC GTTGGCCGAT TCATTAATGC AGCTGGCACG ACAGGTTTCC 19100

CGACTGGAAA GCGGGCAGTG AGCGCAACGC AATTAATGTG AGTTAGCTCA 19150

CTCATTAGGC ACCCCAGGCT TTACACTTTA TGCTTCCGGC TCGTATGTTG 19200

TGTGGAATTG TGAGCGGATA ACAATTTCAC ACAGGAAACA GCTATGACCA 19250

TGATTACGAA TTCGAATGGC CATGGGACGT CGACCTGAGG TAATTATAAC 19300

CCGGGCC 19307

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/86, 7/00, 15/88
A61K 48/00

A3

(11) International Publication Number: WO 96/13597

(43) International Publication Date: 9 May 1996 (09.05.96)

(21) International Application Number: PCT/US95/14017

(22) International Filing Date: 27 October 1995 (27.10.95)

(30) Priority Data:
08/331,381 28 October 1994 (28.10.94) US

(60) Parent Application or Grant
(63) Related by Continuation
US 08/331,381 (CIP)
Filed on 28 October 1994 (28.10.94)

(71) Applicant (*for all designated States except US*): THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA [US/US]; 133 South 36th Street, Philadelphia, PA 19104-3246 (US).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): WILSON, James, M. [US/US]; 1350 N. Avignon Drive, Gladwyne, PA 19035 (US). FISHER, Krishna, J. [US/US]; 4006 Pine Street, Philadelphia, PA 19104 (US). CHEN, Shu-Jen [-/US]; 3901 Conshohocken Avenue, Philadelphia, PA 19131 (US). WEITZMAN, Matthew [GB/US]; 301 S. 19th Street #2A, Philadelphia, PA 19103 (US).

(74) Agents: BAK, Mary, E. et al.; Howson and Howson, Spring House Corporate Center, P.O. Box 457, Spring House, PA 19477 (US).

(81) Designated States: AL, AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, RO, RU, SD, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, LS, MW, SD, SZ, UG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(88) Date of publication of the international search report: 31 October 1996 (31.10.96)

(54) Title: RECOMBINANT ADENOVIRUS AND METHODS OF USE THEREOF

(57) Abstract

A recombinant adenovirus and a method for producing the virus are provided which utilize a recombinant shuttle vector comprising adenovirus DNA sequence for the 5' and 3' cis-elements necessary for replication and virion encapsidation in the absence of sequence encoding viral genes and a selected minigene linked thereto, and a helper adenovirus comprising sufficient adenovirus gene sequences necessary for a productive viral infection. Desirably the helper gene is crippled by modifications to its 5' packaging sequences, which facilitates purification of the viral particle from the helper virus.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgystan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

ERNATIONAL SEARCH REPORT

nal Application No
PCT/US 95/14017

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/86 C12N7/00 C12N15/88 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO,A,95 27071 (BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM) 12 October 1995 see page 15, line 27 - page 16, line 13 see page 19, line 23 - page 24, line 25 see claims 15,16 ---	1-6,13, 19,20,22
X	J.VIROLOGY, vol. 66, no. 2, February 1992, pages 723-731, XP002012398 GRABLE, M. AND HEARING, P.: "cis and trans Requirements for the Selective Packaging of Adenovirus Type 5 DNA"	8-11
Y	see the whole document --- -/--	12,15

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
3 September 1996	20.09.96
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Donath, C

International Application No
PCT/US 95/14017

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PROC.NATL.ACAD.SCI.USA, vol. 89, no. 7, 1992, pages 2581-2584, XP002012528 QUANTIN, B. ET AL.: "Adenovirus as an expression vector in muscle cells in vivo" see the whole document ---	1-7,13, 15-22
Y	BLOOD, vol. 84, no. 5, 1 September 1994, pages 1492-1500, XP002012529 GOODMAN, S. ET AL.: "Recombinant Adeno-Associated Virus-Mediated Gene Transfer into Hematopoietic Progentitor Cells" see the whole document ---	1-7,13, 15-22
Y	BIOCHEM J., vol. 299, no. 1, April 1994, pages 49-58, XP002012399 FISHER, K.J. AND WILSON, J.M.: "Biochemical and functional analysis of an adenovirus-based ligand complex for gene transfer" see page 50, left-hand column, paragraph 1 - page 52, left-hand column, paragraph 1 ---	12,15
T	VIROLOGY, vol. 217, no. 1, March 1996, pages 11-22, XP002012530 FISHER, K.J. ET AL.: "Recombinant Adenovirus deleted of all viral genes for gene therapy of cystic fibrosis" see the whole document -----	1-22

1

ERNATIONAL SEARCH REPORT

Information on patent family members

nal Application No
PCT/US 95/14017

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9527071	12-10-95	AU-B- 2238695	23-10-95

Form PCT ISA 210 (patent family annex) (July 1992)